ANALYSIS OF PROGRAM ACTIVITIES
NATIONAL INSTITUTES OF HEALTH

1956

NATIONAL CANCER INSTITUTE

VOLUME II

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

U.S. National Cancer Institute.

Report of program activities.

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v. 2

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 400
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Biological Studies on the Factors Involved in the Development and Growth of Tumors in Experimental Animals.
PROJECT TITLE

7. H. B. Andervont
PRINCIPAL INVESTIGATOR

8. Harry Y. Canter, M. B. Shimkin and Thelma B. Dunn
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Testicular Tumors

A paper reporting previous work with these tumors will appear in an early number of the Journal of the National Cancer Institute. The findings can be summarized as follows.

Stilbestrol-cholesterol pellets containing 20 percent stilbestrol were inserted subcutaneously into 2-months old strain

(cont.)

(Use reverse and additional pages, if necessary)

Project 400 (cont.)

BALB/c [1] male mice to study the effects of discontinued estrogenic stimulation upon (1) established testicular tumors and (2) the development of tumors in testes of mice exposed to the hormone for limited periods of time.

The estrogen induced a high incidence of interstitial cell tumors of the testis, many of which were bilateral. When pellets were removed from mice with testes measuring 10 to 12 mm. in length, about half regressed to normal size while the remainder continued to enlarge. When mice whose testes had receded to normal received a second pellet, their tumors recurred promptly. In some mice tumors recurred spontaneously.

When mice that had their first pellets removed 5 to 9 months earlier in the absence of any testicular enlargment received second pellets, 50 percent developed testicular tumors within 4 months. Controls receiving their first pellets showed a tumor incidence of but 7 percent within 4 months.

Mice that had their pellets excised 10 to 16 months earlier in the absence of testicular enlargement were killed and their testes examined microscopically. Areas of interstitial cell hyperplasia or changes were found in many of their testes. Testicular tumors arose in some mice from 1 to 12 months after removal of pellets in the absence of testicular enlargement.

These observations indicated the long persistence of interstitial cell changes initiated by stilbestrol and suggested that the changes were irreversible but not sufficient in most instances for further progression unless the mice received a second hormonal stimulus.

Transplantation of testicular tumors that arose in the presence or absence of stilbestrol pellets or those that continued to grow upon removal of pellets resulted in the acquisition of many hormone independent tumors.

These findings suggested that induced testicular tumors of the mouse are of limited dependence upon hormonal stimulation for progression and can achieve independence when the estrogen is removed from hosts carrying primary tumors or in serial passages through estrogenized hosts.

1/ Hereafter called strain C.

During the past year two experiments were completed. One was designed to ascertain the relative susceptibilities of inbred strains of mice maintained in this laboratory to the induction of testicular tumors. The other was performed to determine the susceptibility of various F_1 hybrids derived from strain C and other inbred strains. This experiment was undertaken because a considerable number of strain C mice either die within a few months after insertion of stilbestrol pellets or develop thymic enlargement before they develop testicular tumors. It was hoped that an F_1 hybrid derived from strain C would be more resistant to toxic effects of stilbestrol and to thymic enlargement but sufficiently susceptible to tumor formation to serve as test animals.

Strain Susceptibility

Strain susceptibility was ascertained by subcutaneous insertion of 10% or 20% stilbestrol pellets into 3 to 7 months old mice of 7 strains. The results were as follows.

Strain	% stilbestrol in pellet	No. of mice	No. developed Test. tumors	Average age, mo.
C	20	51	41	11
DBA/2	10	25	2	16
"	20	25	3	16
C3H	20	32	2	17
Y	10	17	0	--
"	20	14	0	--
I	20	15	0	--
C57BL	10	31	0	--
"	20	29	0	--
RIII	10	25	0	--
"	20	20	0	--

It is clear that strain C males were far more susceptible to induced testicular tumors than any other strain.

Hybrid Susceptibility

Six groups of F_1 hybrids and strain C mice as controls were used to test hybrids for susceptibility. All these animals were 2 months old and each received a 20% stilbestrol pellet subcutaneously. The experiment was terminated when all surviving animals were 17 months old. The results were as follows.

Strain or hybrid	No. of mice	No. developed Test. tumors	Average age, months	No. died without Test. tu.	Average age, months
C	25	20	8	5	13
C x C3H	27	10	12	17	14
C x C57BL	38	10	13	28	14
C x DBA/2	24	17	11	7	11
C x I	37	1	11	36	14
C x Y	26	17	11	9	13
C x RIII	37	1	15	36	12

None of the F_1 hybrid group was as susceptible as strain C mice but an appreciable number of C x DBA/2 and C x Y hybrids developed testicular tumors. However, 7 of 24 C x DBA/2 hybrids and 9 of 26 C x Y hybrids died without developing testicular tumors, while 5 of 25 strain C mice did so. Apparently the hybrids are not more suitable than their parent strain C.

Age Susceptibility

Three experiments were undertaken to investigate age differences in susceptibility to induced testicular tumors in strain C. Two were completed. In both, mice of 2, 6 or 12 months of age received 20% stilbestrol pellets. The combined results are summarized below.

Age in months	No. of mice	No. developed Test. tu.	Average time after pellet, months	No. died without Test. tu.	Average time after pellet, months
2	55	42	7.7	13	6.3
6	74	56	7.0	18	4.8
12	79	44	6.7	35	7.4
		Results 10 months after receiving pellets			
2	47	36	6.6	11	4.0
6	65	51	6.6	14	1.6
12	61	42	6.5	19	4.4

The over-all results could be interpreted as suggesting a higher degree of susceptibility of the 12 months old group but it is believed that longer survival of the 2 and 6 months old group accounts for any differences. Sixteen of the 12 months old group died or were in poor health and killed 11 or 12 months after receiving pellets. When only those mice are considered that died or developed tumors within 10 months after receiving pellets, it is seen there is no significant difference between the age groups.

It is of interest that during the first 2 months after insertion of pellets 13 of the 6 months old group, 10 of the 12 months old group and only 3 of the 2 months old group died. This suggests that susceptibility to the toxic effects of stilbestrol increases with age.

In the third experiment mice 2, 4, 6 and 8 weeks old received 10% stilbestrol pellets. This experiment is in progress and tumors are appearing in those 4, 6 and 8 weeks of age. However, of 66 mice in the 2 weeks old group none has developed a testicular tumor and 33 died before 8 months of age. These young mice are very susceptible to the toxicity of stilbestrol.

Persistence of Changes Induced by Stilbestrol

One of the interesting findings of the earlier studies was the prompt occurrence of testicular tumors in mice that received a second stilbestrol pellet 5 to 9 months after removal of their first pellet. An experiment was started to confirm and extend this observation. Eight weeks old strain C males were given 20% stilbestrol pellets subcutaneously. Eight or 16 weeks later the pellets were excised when the testes showed no evidence of enlargement. Twenty-four weeks after excision of pellets the mice received another pellet. As controls, litter mates received their first pellets when the experimental groups received their second pellets. The experiment is in progress but in both series those mice that had carried first pellets are, thus far, developing testicular tumors earlier than the controls. This indicates that 8 or 16 weeks' exposure to hormonal stimulation initiated changes in the interstitial cells of the testes which persisted for at least 6 months as shown by the early appearance of testicular tumors.

Recurrence of Testicular Tumors

Another interesting observation during the earlier work was the regression of testicular tumors after excision of pellets and the prompt recurrence of these tumors after administration of another pellet. This phase of the investigation is receiving much attention but progress is slow because only 50% of tumors regress and 4 to 6 months elapse before a second pellet is implanted. It has been found that when pellets were removed from mice with testicular tumors averaging 10 mm in length about 50% of the tumors regressed. Such mice were kept for 6 months after regression of their tumors and about 50 percent of the animals developed spontaneous recurrences - that is, without exposure to a second pellet. Those mice in which tumors did not recur spontaneously within 6 months received second pellets and their tumors recurred promptly.

The above mice in which tumors recurred spontaneously were permitted to carry pellets for an average of 3 weeks after appearance of tumors. In another series of mice pellets were removed as soon as the testes were definitely enlarged - 9 to 10 mm. in length. In this series of animals the tumors did not recur spontaneously within 6 months. (Information is not available on the response of these mice to second pellets). The average testis size in this series was about the same as that in the mice with recurrences. At the present time the only obvious reason for the difference in rates of recurrence is the exposure to pellets for an average time of 21 days.

If this can be confirmed it will establish a critical period of 3 weeks exposure to stilbestrol in which reactions are initiated which eventuate in the recurrence of testicular tumors.

Breast Tumors

Progesterone Studies

In the last report preliminary findings were presented of an experiment in which stilbestrol and progesterone were administered to agent-free strain C3H females. This experiment is finished and the tumors have been diagnosed microscopically. The results are as follows.

Group	No. of mice	No. with mammary tumors	Average age, in months	No. dead without mammary tumor	Average age at death, in months
10% stilbestrol pellet	41	5	20	36	24
10% stilbestrol pellet plus progesterone	39	27	20	12	24
20% stilbestrol pellet	37	7	18	30	22

This experiment is being repeated and extended by using castrate males as well as intact females. It also led to the initiating of a few exploratory experiments in which the effects of stilbestrol alone are compared to the combined effects of stilbestrol and progesterone.

One experiment is completed in which 6 months old agent carrying virgin strain C3H females were used. Of 27 that received stilbestrol alone, 25 developed tumors at an average age of 10 months; 2 are living at 13 and 14 months of age. Of 26 that received stilbestrol and progesterone, 24 developed tumors at an average age of 9 months; 2 died tumor free when 11 and 12 months old. This finding in older virgin strain C3H females was sufficiently encouraging

to start another experiment with 2 months old test animals.

In another experiment still in progress, 2 months old agent carrying virgin strain C females were used. Of 19 receiving stilbestrol alone, 4 have developed tumors, whereas of 19 receiving both compounds, 11 have developed tumors.

Another experiment in progress consisted of breeding strain C agent carrying females. Of 22 that received no treatment 5 have developed tumors. Of 16 that received progesterone only, 10 have developed tumors.

In another experiment strain C males received the hormones. To date it appears that progesterone exerts an inhibitory influence upon the induction of testicular tumors in these animals. For example, of 36 mice that received 20 percent stilbestrol pellets when 2 to 4 months of age, 11 have developed testicular tumors. Of 36 that received both compounds only 2 have done so.

Agent Studies

During the year a series of experiments were started to ascertain whether the mammary tumor agent from wild mice increased in activity after 20 passage generations in inbred strain C mice.

A presumably agent free line of wild mice was started and these animals, together with members from the wild colony, are being tested for the presence of the agent by two procedures. (1) By foster nursing susceptible agent free inbred mice. (2) By backcrossing to males of a strain known to be relatively susceptible to the wild mouse agent.

Efforts to induce mammary tumors in wild mice have not been successful even when they received a combination of stilbestrol and progesterone. Wild mice are very susceptible to the toxic effect of stilbestrol and, because of their wild nature, large numbers cannot be used in any one experiment.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 400
SERIAL NUMBER

12. BUDGET DATA:

ESTIMATED OBLIGATIONS			MAN YEARS		
DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
$64,340	$21,170	$85,510	2	6	8

FY'57

BUDGETED POSITIONS			PATIENT DAYS
PROF	OTHER	TOTAL	
2	6	8	0

FY'57

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 400
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Bang, F. B., Andervont, H. B. and Vellisto, I.: Electron microscopic evidence concerning the mammary tumor inciter. II. An electron microscopic study of spontaneous and induced mammary tumors of mice. Bull. Johns Hopkins Hospital, 98: 287-308, 1956.

Andervont, H. B.: Problems before the Association. Cancer Research, 16: 471-478, 1956.

Andervont, H. B. and Edgcomb, J. H.: Responses of seven inbred strains of mice to percutaneous applications of 3-methylcholanthrene. J. Nat. Cancer Inst. 17: 481-495, 1956.

Andervont, H. B., Shimkin, M. B., and Canter, H. Y.: Effect of discontinued estrogenic stimulation upon the development and growth of testicular tumors in mice. J. Nat. Cancer Inst. (in press).

Andervont, H. B., and Dunn, T. B.: Studies of the mammary tumor agent carried by wild house mice. Acta U. Internat. Contre Canc. (in press).

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 401
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cellular Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The relation between cell chemistry and cell structure
PROJECT TITLE

7. E. L. Kuff, G. H. Hogeboom (deceased July, 1956)
PRINCIPAL INVESTIGATOR

8. J. L. Irvin (Guggenheim Fellow) and Dr. A. J. Dalton
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objective: To arrive at an understanding of the structural organization of normal and malignant cells in terms of their biochemical constituents. It is now generally recognized that the spatial relationships that exist between biochemical components within cells may influence - and indeed control - many of the metabolic functions of the cells. The information upon which this conclusion is based has come largely from the study of the distribution of enzymes and other compounds among the particulate structures (nuclei, mitochondria, etc.) and the soluble fractions released by the mechanical disruption of cells. Studies at this level of organization are, of course, not yet complete and will be continued. In addition, however, and because of the apparently subtle nature

(Use reverse and additional pages, if necessary)

of the differences between normal and malignant cells, it has become important to study increasingly finer details of structural and biochemical organization. This is being done in two ways; (1) high-resolution electron microscopy of intracellular material isolated by precise methods of centrifugal fractionation and characterized biochemically; and (2) morphological and biochemical study of the products obtained when isolated intracellular structures are themselves disrupted by controlled physical or chemical means.

Methods employed: A variety of centrifugal methods are employed in this laboratory for the isolation and characterization of intracellular particulates. The development of these methods has been described in previous Annual Reports and they may be simply listed here: (1) differential centrifugation; (2) density gradient fractionation; (3) packing of particles under sufficient centrifugal force to cause layering of different particle types; (4) size distribution analysis employing a high speed swinging bucket rotor. Methods 2 and 4 may be satisfactorily employed in the study of particles ranging in size down to that of serum albumin. The optical centrifuge is available for smaller molecules and will presently be improved in analytical specificity by the installation of quartz optics. The preparation of isolated fractions for electron microscopy has been improved to the point where it is now possible to fix, embed, and observe fractions composed of materials of molecular dimensions.

Results: (1) Ribonucleic acid (RNA) content and electron microscopy of isolated mitochondria. Rat liver mitochondria isolated in sucrose by the packing method were found still to contain appreciable quantities of RNA. Electron microscopy of these preparations revealed that contamination with other structural elements of the cell was negligible and indicated that RNA is indeed a constituent of the mitochondria themselves. This conclusion bears on the question of the possible self-duplicating nature of the mitochondria and the role of RNA therein.

(2) Studies on the "soluble RNA" of liver cells. Sedimentation studies of rat liver homogenates show that the truly soluble RNA represents about 8 percent of the total RNA of the normal liver cell and is distinctly separate in particle size from other RNA. In crude supernatant preparations, it forms a relatively sharp sendimentation boundary with an $s_{20,w}$ between 3.5 and 5, depending on the nature of the solvent. Work in other laboratories has indicated that the soluble RNA may be protein-bound. In our experiments a major protein sedimentation boundary occurs in precisely

the same portions of the centrifuge tubes as does the RNA boundary and is thought to be formed by the s = 3.6 class of soluble liver proteins discovered by Sorof and found by him to contain the bulk of the soluble protein-bound aminoazo derivatives resulting from the feeding of various hepatocarcinogens. Work is under way to determine whether the soluble RNA is actually bound to one or several members of this class of proteins.

(3) Incorporation of radioactive glycine into protein and RNA of liver cell fractions (in collaboration with Dr. Irvin). This project is not complete. Rats were injected intraperitoneally with C^{14}-labelled glycine, sacrificed at varying intervals thereafter, and homogenates of the perfused livers fractionated by several methods. Electron microscopy and enzymatic assays were employed to characterize the fractions. Proteins and the purine bases of RNA were isolated from the fractions (the latter by chromatographic methods developed during the course of the study) and their specific activity of C^{14} determined. As far as we are aware, this is the first time that protein and RNA synthesis has been studied simultaneously in fractions morphologically defined by electron microscopy.

Since the C^{14} assays are not complete, it would be premature to discuss the results of the study in terms of RNA and protein synthesis. It should be noted, however, that the work has already resulted in considerable clarification of the question of RNA distribution in homogenates. It was found that a group of particles (termed "ultramicrosomes") could be differentiated from the main bulk of the microsome fraction on the basis of smaller size (15-20 mu), greater RNA nitrogen ratio, and more rapid rate of incorporation of glycine-C^{14} into the RNA purines. Electron microscopy revealed that the ultramicrosomes corresponded in appearance to the so-called "ribonucleoprotein" particles first described by Palade in intact cells. We have also observed similar particles attached to the surface of the larger vesicular elements that compose the main microsome fraction. The situation in homogenates thus appears to correspond to that seen in intact cells, where the small particles are found both free in the cytoplasmic matrix or attached to the membranes of the ergastoplasm. Observed differences in the rates of uptake of glycine-C^{14} into RNA and protein support the conclusion that the fractions were not artifacts but rather were composed of elements that existed as separate entities within the cells. The ultramicrosomes were found to be lacking in the two enzymes characteristically associated with the whole microsome fraction - DPNH-cytochrome c reductase

and glucose-6-phosphatase.

(4) Identification of ferritin in liver homogenates and tissue sections. In the course of the above study, a type of particle not previously observed either in homogenates or intact cells was found to be concentrated in a cell fraction isolated at high centrifugal speeds. The structure of the particle (see Annual Report of Dr. A. J. Dalton) suggested that it might be the iron-storage protein, ferritin. A study of the quantitative distribution of total iron (analyses by Dr. Greenfield and Price) among all of the cytoplasmic fractions showed that the iron content of the fractions paralleled the frequency of occurrence of the particles in the fractions. Known rat liver ferritin was isolated centrifugally from a mixture of ferritin and catalase prepared by Dr. Price and found to correspond in appearance under the electron microscope with the particles seen in the cell fractions. High-resolution electron micrographs of rat and mouse liver revealed the same particles in situ. It is considered established that they represent ferritin. These results will be submitted for publication shortly.

As a result of this work, preliminary studies have been undertaken in collaboration with Drs. Price and Greenfield regarding the possibility of separating catalase and ferritin from one another by a relatively simple centrifugal method. The results indicate that density gradient fractionation may provide catalase at a purity comparable to that obtained by chromatographic techniques. A very pure ferritin is easily obtained.

Significance for cancer research: This program is based upon the supposition that increased knowledge of the spatial and functional organization of intracellular materials will provide additional points of attack in the search for exploitable differences between normal and malignant cells. The importance of the morphological study of isolated cell constituents was pointed out in the previous Annual Report. Interest in the physical properties, intracellular distribution, and metabolism of RNA arises from the pivotal role that this material is thought to play in controlling protein synthesis. The ferritin studies, while a by-product of other work and unrelated to cancer research at present may lead to the development of methods useful for the study of iron metabolism in cancer-bearing animals.

Proposed course of the project: (1) the correlated biochemical and morphological study of intracellular particulate materials will be continued.

(2) The incorporation studies described above will be completed and it is tentatively planned to undertake a similar investigation of transplantable hepatoma.

(3) Study of the characteristics of the low molecular weight soluble RNA in homogenates will be continued, in view of many reports in the literature of high levels of this material during states of rapid growth.

(4) Work on ferritin and other iron-rich intracellular components (e.g., hemosiderin granules) will be continued, particularly with regard to their relationship to other structures within the cell. A collaborative project with Drs. Price and Greenfield for the development of methods for the centrifugal purification of catalase and ferritin is under way.

(5) If time permits, it is hoped to reinstigate sedimentation studies of the soluble components released upon physical disruption of the cell particulates of normal and malignant tissues (see Annual Report #401 of last year, prepared by Dr. Hogeboom).

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 401
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$16,080	$5,300	$21,380	1	1	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 401

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Kuff, E. L., Hogeboom, G. H., and Dalton, A. J.: Centrifugal, biochemical, and electron microscopic analysis of cytoplasmic particulates in liver homogenates. J. Biophys. Biochem. Cytol. 2: 33-54, 1956.

(2) Hill, B. R., Kuff, E. L., and Hogeboom, G. H.: Sedimentation behavior of serum lactic dehydrogenase. Proc. Soc. Exp. Biol. Med., 92: 430-433, 1956.

(3) Kuff, E. L., and Hogeboom, G. H.: Sedimentation and biochemical characteristics of cytoplasmic particulates in enzymes: Units of biological structure and function. pp. 235-251 (1956), Academic Press, New York.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

By invitation, Drs. Hogeboom and Kuff attended the 1956 Canadian National Cancer Congress and Dr. Hogeboom addressed the Congress on the role of cytochemistry in cancer research.

Form No. CRP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. [illegible]
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cellular Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Oxidative metabolism in various cellular [illegible] and tumor tissue.
PROJECT TITLE

7. Ruth K. Kielley
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To study the in vitro effect of various carcinogens on oxidations occurring in mitochondria and in homogenates of mammalian liver and to learn the mechanism of action underlying these effects.

Methods employed: Homogenates and mitochondria were prepared from normal rat liver and were used as the enzyme source for testing the effect of various fluorene carcinogens on the oxidation of d-isocitrate, l-glutamate, [illegible]-ketoglutarate, d,l-3-OH butyrate and succinate by the usual Warburg manometric method. The extent of phosphorylation accompanying these oxidations was also measured. The mechanism of action of the fluorene carcinogens

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was studied by means of experiments with extracts of mitochondria. The effect of the carcinogens was studied directly on the glutamic dehydrogenase activity of the extracts.

Principal Findings: The principal findings may be summarized as follows: (1) In a phosphorylating mitochondrial system, glutamate oxidation as well as the accompanying phosphorylation was markedly inhibited by the carcinogen, N-2-fluorenylacetamide. Glutamate oxidation was inhibited to a significantly greater extent (90 per cent) than any other DPN-linked oxidation studied. Succinate oxidation was not inhibited. A rough correlation was observed between the degree of inhibition of glutamate oxidation and the potency of the fluorene derivatives as carcinogens when administered to rats. The carcinogen also inhibited glutamate oxidation in rat liver homogenates, but the extent of inhibition was less than that observed with mitochondria. (2) In further studies on the nature of the inhibition of glutamate oxidation by the carcinogen, N-2-fluorenyl acetamide, it was shown that mitochondria exhibited considerable variation in their response to the carcinogen. The variability appeared to be related to the state of binding of DPN in the mitochondria which in turn appears to be associated with the strain of rat. It was shown by experiments with 2,4-dinitrophenol that progressive "uncoupling" of the mitochondria leads to a progressive release of the inhibition caused by the carcinogen. From studies on the mechanism of action of the carcinogen, it was found that the carcinogen has a direct action on the glutamic dehydrogenase of mitochondria. The mechanism of inhibition was shown to involve competition of the carcinogen with DPN for the glutamic dehydrogenase of mitochondria. The carcinogen is an effective inhibitor only on the "native enzyme" and not on crystalline glutamic dehydrogenase and appeared to have a considerably greater affinity for the enzyme than did the natural cofactor.

Significance to cancer: The implications from the findings of the studies on inhibition of glutamate oxidation by the carcinogen, N-2-fluorenylacetamide, are those relating to the mechanism of carcinogenesis. According to the views of Warburg the primary cause of cancer is an irreversible injury of respiration. From this broad generalized statement, one might ask is there a specific step in the respiration process whose injury creates a condition that is unique for the development of malignancy? The results of the present work offer some interesting thought and speculation about metabolic conditions induced by carcinogens to which the cell might become adapted before actual transformation to

a cancer cell. A suppression of glutamate oxidation could conceivably result in a high level of glutamate in cells since one of the major pathways for transferring this amino acid to the carbohydrate cycle would be blocked. In the presence of a sustained abnormally high level of glutamate, cellular mutation, dedifferentiation or whatever term is liked, might occur involving synthesis of protein in a slightly altered manner, perhaps with the inclusion of a few more glutamate molecules. This step brought about initially by an inhibition of glutamate oxidation might represent in its simplest form the transformation of a normal cell to a cancer cell. This, in essence, is the rationale of thinking in the problem presented in this report.

Proposed research: At the present time, work is in progress on the effect of various types of carcinogens on glutamate oxidation in bone marrow and in isolated mitochondria of several different strains of mice. Some preliminary results indicate that o-aminoazotoluene markedly inhibits glutamate oxidation in mitochondria isolated from livers of strain A mice (Rat liver mitochondria previously studied were not inhibited by this carcinogen.) whereas 3'Me-DAB, the acetylated derivative of o-aminoazotoluene and 4'-aminoazotoluene exerted little or no inhibition on these mitochondria. It seems particularly appropriate and important at this time following the initial discovery that N-2-fluorenyldiacetamide acts as a powerful inhibitor of glutamate oxidation in rat liver mitochondria to try to establish whether or not this effect is a general phenomenon associated with the carcinogenic process which can be correlated with the susceptibility of certain animals to a particular kind of carcinogen in the development of tumors. To arrive at a well founded conclusion, it is essential that the problem be approached from several directions involving extensive in vitro and in vivo studies. Although it is desirable from the nature of the problem to cover as wide a range of carcinogens and animals as possible, in view of the developing complexity of the problem and the lack of any technical assistance to carry on the routine analytical work, it seems best to limit the efforts of the one investigator to only a select few carcinogens and perhaps two strains of mice that show marked differences in susceptibility to a particular carcinogen. Since it is likely that all carcinogens do not act directly on glutamate oxidation, it is proposed for the future that some in vivo studies on the levels of glutamate and glutamine in tissues and blood of animals receiving carcinogens be carried out in order to test the fundamental idea that glutamate oxidation may be the key reaction in respiration that is impaired.

Form No. CRP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. _____
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$8,040	$2,640	$10,680			0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	0	1	0

13. BUDGET ACTIVITY:

RESEARCH	☐	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. LO4
(SUBJECT NUMBER)

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Kielley, R. K.: Reduction of 2,4-dinetrophenol and other nitrogen compounds by liver xanthine oxidase. In Inorganic Nitrogen Metabolism, Flavo Proteins. (McElroy, ed.) Baltimore, Johns Hopkins Press, 1956, p. 205.

(2) Kielley, R. K.: Inhibition of glutamate oxidation by fluorene carcinogens in a phosphorylating mitochondrial system. Biochimica et Biophysica Acta, 21, 574 (1956).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. CRP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 406
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cellular Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Electron Microscopy of normal and malignant cells.
PROJECT TITLE

7. A. J. Dalton
PRINCIPAL INVESTIGATOR

8. Marie D. Felix
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: Several separate studies, both individual and collaborative, are under way at the present time. The specific aim of each study is different but each one was chosen because it satisfied the primary objective of the unit, that of applying the high resolving power of the electron microscope to problems relating to the normal and abnormal growth, function, and differentiation of cells.

Since these studies vary greatly in detail and are at different stages of development, they will be listed separately with a descriptive title, followed by the name or names of

(Use reverse and additional pages, if necessary)

collaborators, major findings, significance and proposed course.

(1) Study of the Golgi complex in normal and malignant cells. With Marie D. Felix.

This study has elucidated the varied structure of this cell organelle and has resulted in further evidence in support of the view that one of its primary functions in both absorption and secretion is the control of intracellular water balance. The concentration of water within vacuoles of the complex, both during maturation of precursors of secretory droplets and in the absorption by cells of substances from the external environment have been clearly indicated. A hydrophyllic phospho-lipid within the complex is thought to be involved in this activity and is considered to be responsible for the classical Golgi reaction, the reduction of osmium or silver within the complex. The simplified form of the complex in anaplastic tumor cells and the frequent failure to obtain the classical Golgi reaction in such cells is negative evidence in support of the above hypothesis (Ref. 1).

This study was part of a larger one still going on but which is now receiving and in the future will receive less attention - that of a comparison of the fine structure of normal and malignant cells. The general conclusions from this earlier study have been that no qualitative differences exist but that differences in the number, size, and internal structure of mitochondria and in the degree of organization of the Golgi complex may be found in specific instances of comparison (Ref. 2 and 3).

The comparison of macrophages with mesothelial cells has resulted in the demonstration of a real difference in their fine structure. Macrophages possess a more electron lucent ground substance in the cytoplasm and much larger mitochondria. It has also been shown that mesothelial cells are incapable, under the conditions of the experiment, of engulfing particles larger than 0.1 u. These observations are of some significance in relation to identification of cell types in the peritoneal cavity. These two cell types have been confused with one another frequently in the past. (Ref. 4).

(2) Study of the Rous agent and Rous sarcoma. (with W. R. Bryan.)

This study was initiated in order to take advantage of Dr. Bryan's quantitative biological test for Rous virus filtrate potency in an attempt to relate the possible presence of virus-like particles in filtrate induced tumors with the known potency of the filtrate. To date, of 15 tumors known to have been induced with a filtrate of high potency, 14 have been demonstrated to contain a virus-like particle of consistent size (400-500 $\mathring{A}$) and structure. In addition, in 3 of these tumors, cells were found containing numerous smaller particles (200-300 $\mathring{A}$) in clumps throughout the cytoplasm. Whether these smaller particles represent a stage in the development of the virus or a reaction of the cell to the presence of the virus is at present undetermined. In none of the 8 low potency strain tumors examined (tumors capable of being transferred by cells only, not filtrate) neither of these particles have been identified. The growing tendency to accept the concept of the ubiquitous presence of virus particles in normal and malignant cells lends considerable import to this study since it is one of the few feasible ways of demonstrating a significant relationship between a particle and the existence of the malignant state. Dr. F. Haguenau, recently appointed as a visiting scientist, will be working on one aspect of this study - an attempt to determine the life cycle of the Rous agent.

(3) The electron microscopy of He La cells grown in deficient media. With H. Eagle.

Examination of HeLa cells grown in media free of (1) tyrosine, (2) glutamine, (3) folic acid, and (4) glucose, had indicated that apparently specific alterations in mitochondria (tyrosine and folic acid deficiencies) and nucleoli (glutamine deficiency) resulted. More recent studies of squamous epithelial cells from Dr. Earle's laboratory by Dr. Menefee suggest that these changes in HeLa cells were not specific for the vitamin and amino-acids involved. (Dr. Menefee's results will be reported separately.)

(4) The application of electron microscopy to the problem of embryonic induction (With Dr. Grobstein).

Earlier studies by Dr. Grobstein with ordinary light and phase contrast microscopy suggested but did not prove that the induction of kidney tubule formation by developing nervous tissue did not require actual cell contact. Analysis of nephrogenic mesenchyme-filter-neural tissue preparations with the electron microscope indicated that induction may occur in the absence of cell contact between the two tissues. A paper describing these results is in press.

(5) Analysis of hen oviduct homogenates with the electron microscope. (With Drs. Hendler and Glenner).

Chemical and biochemical studies by Dr. Hendler indicated that fractions of homogenates of the glandular portion of the hen oviduct obtained by differential centrifugation did not show the type of activity characteristic of fractions of homogenates of liver tissue obtained as a result of the application of the same centrifugal speeds. Examination of hen oviduct fractions with the electron microscope demonstrated that the ergastoplasm or basophilic component characteristically present in the so-called "microsome" fraction of centrifuged homogenates of liver tissue is thrown down almost entirely at very low speeds. This explained the difference in the chemical and biochemical analyses of the fraction from the two tissues. From our point of view, the most significant finding was obtained from examination of sections of intact oviduct. Electron micrographs showed that the ergastoplasm is the primary site of albumin synthesis. Neither mitochondria nor the Golgi complex appeared to be involved in this activity. A manuscript describing these results has recently been submitted for publication.

(6) Identification of ferritin in cells of the liver and spleen. (With Dr. Kuff).

The chemical and biochemical results and the technical aspects of the centrifugation methods employed in this collaborative study will be described separately by Dr. Kuff. At this point, it needs only to be mentioned that the present findings are the tangible results of a continuing study aimed at a more detailed analysis of fractions obtained from differential centrifugation of homogenates of hepatic tissue. Electron micrographs of thin sections of pellets obtained after application of very high centrifugation speeds for relatively

long periods demonstrated the presence of numerous previously unobserved, very small (50 $\mathring{A}$) but electron dense particles. Mixed as they were with other larger particles, each small particle could be seen to be surrounded by a distinct electron lucent halo 50 $\mathring{A}$ wide, giving the whole particle a diameter of approximately 150 $\mathring{A}$. Particles of the same size and morphology were then found in electron micrographs of thin sections of parenchymal hepatic cells and in macrophages of the spleen. For reasons given by Dr. Kuff these particles have been identified as ferritin. This is the first time to our knowledge that a macromolecule of known chemical composition has been identified both in fractions of centrifuged homogenates and in sections of intact cells. In addition to the significance implicit in the fact that ferritin is importantly involved in iron storage and transport, these results suggest that the continued application of these techniques to problems of cell chemistry will prove to be more fruitful than many workers in the field have thought possible.

(7) The electron microscopy of a series of primary and transplanted lymphoid tumors. (With Drs. Law and Potter).

This study is a long-term one and cannot be expected to produce immediate results. However, the implications involved in its eventual completion are of considerable importance. The first question involved is that of the virus etiology of lymphoid tumors and this is complicated by the presently accepted view of the ubiquity of virus particles in normal animal cells. The second question is, whether once induced, does the retention of the malignant state require the continued presence of virus? The essentially qualitative character of thin section work in electron microscopy makes this study a difficult and time consuming one. However, it is felt that the potentialities involved make it well worth pursuing. To date, 4 transplanted tumors have been studied. Three of these have given no evidence of the presence of virus-like particles while the fourth has been found positive. No primary tumors have been examined so far.

(8) The electron microscopy of two "single cell" clones derived from strain L. (With Dr. Sanford and Myra Embrey).

Earlier examination of the "malignant" and "benign" clones of strain L was complicated by the presence of

virus-like particles both within and on the surface of many of the cells of the "benign" clone. Similar particles were not found in cells of the "malignant" clone in vitro but were found subsequently in vivo. Recently two new single cell clones derived from the "benign" strain have been examined. These have been grown on a synthetic medium. No evidence of virus-like particles has been found in either clone but distinct differences in the fine structure of the cells of the two clones have been noted. In cells of the relatively "malignant" strain, the mitochrondria are few in number, large and relatively electron lucent. In addition, membranes of the ergastoplasm are rarely encountered. In cells of the relatively "benign" strain the mitochondria are numerous, small and relatively electron dense. Membranes of the ergastoplasm are relatively numerous. These differences were found consistently in the many cells examined but their significance is at present unknown. The implications of the finding of virus-like particles in the earlier studies needs further clarification.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 406
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$40,210	$13,270	$53,480	1	4	5

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	4	5	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 406
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Dalton, A. J. and Felix, M. D.: A comparative study of the Golgi complex. J. Biophys. Biochem. Cytol. 2: 79-84, 1956

(2) _____: The electron microscopy of normal and malignant cells. Annals N. Y. Acad. Sc. 63: 1117-1140, 1956.

(3) Selby, C. S., Biesele, J. J., and Grey, C. E. (With discussion by A. J. Dalton): Electron microscope studies of ascites tumor cells. Annals N. Y. Acad. Sc. 63: 767-773, 1956.

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR 1956:

(1) Organizer and chairman of symposium on "Membrane systems of cells" given at the annual meeting of the Electron Microscope Society of America, Madison, Wis., Sept. 1956

(2) Invited to five expenses-paid seminars on general subject of electron microscopy of animal cells. Dept. of Anatomy, U. of Penn. Med. School and Dept. of Zoology, Duke U.

(3)Invited to be an associate editor of a new European Journal of Electron Microscopy.

(4) Invited to write chapter on "The Golgi Apparatus and Secretory Granules" in "The Cell" to be edited by Mirsky and Brachet.

(5) Invited to be visiting Research Professor, Dept. of Anatomy, School of Med., U. of Minnesota - academic year of 1957-58.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 407
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cellular Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Precursors of Deoxyribonucleic Acid (DNA) in Normal, Regenerating, and Tumor Tissues.
PROJECT TITLE

7. Dr. Walter C. Schneider
PRINCIPAL INVESTIGATOR

8. Dr. Jean Rotherham (since August, 1956)
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To isolate and identify the deoxyribose compounds other than DNA present in normal and cancer tissues and to determine the metabolic pathways in which they are involved.

Methods: The deoxyribose containing compounds are separated from DNA and most of the rest of the tissue by making cold acid extracts of the tissues (obtained under anesthesia and frozen in liquid nitrogen). The deoxyribosidic compounds are detected and measured microbiologically with the organism L. acidophilus R-26 and are isolated by means of ion exchange and paper chromatography and paper electrophoresis. Identification of the isolated

(Use reverse and additional pages, if necessary)

compounds is based on spectral, chromatographic, and chemical behavior.

Major findings: The widespread distribution of deoxyribosidic compounds other than DNA in normal and tumor tissues of the rat and the mouse and the separation and identification of the deoxyribonucleosides present in rat liver and hepatoma was reported previously. It was also noted that deoxynucleotide-like compounds accounted for a large proportion of the total acid soluble deoxyribosidic compounds in the rat hepatoma and in regenerating liver. Since normal rat liver did not contain these compounds in appreciable amounts, it was concluded that deoxynucleotides were especially important in growing tissues. The studies of the past year have accordingly dealt with attempts to identify these compounds.

Experiments on the actual isolation and purification of the deoxynucleotides present in regenerating liver and hepatoma have been only partially successful. This has been due largely to technical difficulties encountered in recovering these compounds from ion exchange columns. One of the nucleotides present in the hepatoma extract was, however, purified about 100 times, but identification was not possible because the small amount of material remaining precluded further purification.

Exploration of the growth requirements of the organism used for the detection of the deoxyribosidic compounds has shed considerable light on the nature of the tissue deoxynucleotides. These studies have shown that the organism grows equally well on any natural deoxynucleoside or deoxymononucleotide. In collaboration with Dr. R. L. Sinsheimer of Iowa State College, it was found that the organism was able to grow equally well on either of two pure deoxydinucleotides while experiments with Dr. R. L. Potter of the University of Michigan have demonstrated that the organism cannot grow on phosphorylated deoxynucleotides such as thymidine or deoxycytidine di- and triphosphate. These findings indicate that the tissue deoxynucleotides are either mono- or polynucleotides.

Since the organism cannot grow on the phosphorylated deoxynucleotides, some other method was needed which could determine whether these compounds were present in tissues. Such a method has now been developed. It consists simply of incubating a tissue extract with purified intestinal phosphatase (supplied by Dr. Heppel) to hydrolyze the phosphorylated deoxynucleotides to nucleotides or nucleosides. These compounds are then measured microbiologically.

Preliminary assays have shown that considerable amounts of phosphorylated deoxynucleotides are present in rat thymus, regenerating liver and hepatoma, in addition to the deoxynucleosides and deoxynuclectides previously detected.

Significance to cancer research: The fundamental problem facing cancer research is the determination of why cancer cells in the malignant stages multiply constantly while the reproduction of normal cells is restricted and controlled. The study of DNA synthesis is a fundamental approach to this problem because DNA is an essential constitutient of the chromosomes, and is the only known cellular constituent whose duplication, prior to cell division, is obligatory. The studies above are aimed at obtaining information on the mechanism of DNA synthesis and offer hope that the control of cell division and tumor growth can be placed on a rational basis.

Proposed course: Efforts to isolate and identify the deoxynucleotides in regenerating liver and in tumors are in progress and will continue. Using the new assay procedure, which permits the simultaneous determination of deoxynucleoside, deoxynucleotide, and phosphorylated deoxynucleotide, concentration, a comprehensive survey of various normal and tumor tissues will be made. Studies on the metabolism of deoxyribose compounds are contemplated, and studies on the ability of the test organism to utilize deoxypolynucleotides larger than dinucleotides will be pursued with the collaboration of Dr. Sinsheimer.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 407
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$16,080	$5,300	$21,380	1	1	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications [illegible] SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Schneider, W. C.: Structural factors in metabolic regulations. Proc. of the Third International Congress of Biochemistry. Conferences and Reports. p. 305, edited by C. Liebecq, Liege, 1956.

(2) Schneider, W. C. and Hogeboom, G. H.: Biochemistry of cellular particles. Ann. Rev. Biochem. 25: 201, 1956.

(3) Schneider, W. C., Striebich, M. J., and Hogeboom, G. H.: Cytochemical studies. VII. Localization of endogenous citrate in rat liver fractions. J. Biol. Chem. 222: 969, 1956.

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Invited to serve as chairman of the section on "Particulate Chemistry of Cancer Cells" at the First Pan American Cancer Cytology Congress in Miami, April, 1957.

(2) Invited to prepare a comprehensive, critical review on mitochondrial metabolism to be published in Advances in Enzymology, 1959.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 408
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cellular Biology
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on lymphocytic tumors.
PROJECT TITLE

7. Emma Shelton
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To investigate the biological mechanisms which may be involved in the dissemination of the cells of lymphocytic leukemias in the mouse.

Methods: The cells of three transplantable lymphocytic leukemias of the mouse, namely, L#1, L#2, and L#1210, show a different capacity to invade tissues located at some distance from the site of inoculation of the tumor into the host. The survival time of the host is correlated with the extent of this invasion. There can be no simple explanation for this difference in behavior of these cells; the factors concerned may be very complex ones involving the metabolism and growth rate of the cells. It was felt

(Use reverse and additional pages, if necessary)

that a simple starting approach to the problem would be to investigate the capacity of the cells of these tumors to pass through the pores of inert membranes. Since the membranes are not attacked by the enzymes of the cell, the passage of the cells through the pores would be accomplished by mechanical rather than enzymatic mechanisms.

"Diffusion chambers" of two types were used.

(1) Simple chamber: A circle of lucite, 1 mm. thick, on both sides of which are cemented cellulose membrane filters of porosities ranging from 0.1 to 0.5 microns.

(2) Double chamber: Two circles of lucite between which are cemented membranes with porosities ranging from 5 microns to 0.8 microns. The double chamber is closed by cementing on the outside of each lucite circle a membrane with a porosity of 0.1 microns, through which cells cannot pass.

Two types of experiments were performed.

(1) Simple chamber: Known numbers of cells of the lymphomas in the ascites form were put into the chambers and the chambers inserted into the peritoneal cavity of mice. The passage of the cells through the pores of the membranes was determined by the appearance of the tumor cells in the peritoneal fluid of the host.

(2) Double chamber: Known numbers of cells were placed on one side of the center membrane of the chamber and the ability of the cells to pass through the center membrane into the other (empty) side of the chamber was determined by microscopic examination of the fluid on the empty side at various intervals after the chamber was placed in the peritoneal cavity of the mouse. In any given experiment the same number of cells of each tumor were put into the chambers.

Major findings: (1) Simple chamber: The "percent escape" of the cells from these chambers could not be correlated with the porosity of the membrane nor the type of tumor. It was obvious from the results that the escape of the tumors was due to imperfect sealing of the membranes onto the lucite ring. Those cells which did not escape, survived in the chambers for periods up to 9 months and inoculation of these cells into the host produced a tumor at the injection site. Some chambers are still in the host.

(2) Double chamber: The passage of the cells through the center membrane of these chambers appears to be correlated with the membrane porosity and the type of tumor. Results can be summarized as follows:

a) Membrane pore size, 0.8 microns: After 25 days, no cells of any of the tumors were found to pass through the membrane.

b) Membrane pore size, 1.2 microns: L1210 (most rapidly and widely disseminated in host). Cells pass through pores of membrane between 10 and 13 days after implantation of chamber in mouse.

L#2 (shows intermediate capacity to disseminate in host). No tumor cells were observed to pass through the membranes at 10 days, but between 11 and 13 days, 80 percent "escape" occurred. The number of cells appearing on the empty side of the chamber was less than that seen with L1210.

L#1 (least disseminated in host). Thus far, no cells of this tumor have been observed to pass through the pores of this membrane after 13 days in the host.

c) Membrane pore size 3 microns and 5 microns: Results thus far obtained show that the cells of all three tumors pass through the pores of these membranes within 3 to 4 days after implantation of the chamber into the host.

Significance to cancer research: The nature of the invasive properties of tumor cells is little understood. These experiments are being carried out in the hope that they will shed some light on the phenomenon.

Proposed course of the project: It is hoped that future work can show some reasons why the cells of these lymphomas show different capacities for passing through the 1.2 micron pores of membrane filters. It is possible that factors such as growth rate, speed and mode of cell locomotion and life span of the cells may be involved. The future course of this project is aimed at examining these factors.

Form No. ORP-1
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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 408
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$10,710	$3,520	$14,230	2/3	2/3	1-1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2/3	2/3	1 1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No items for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 409
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cellular Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on liver carcinogenesis in the mouse
PROJECT TITLE

7. Emma Shelton
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To study the changes occurring in the parenchymal cells of the liver of the mouse prior to the formation of a tumor; and to develop methods for studying these changes.

Methods: Nutritional, biochemical, histochemical and histological.

Major findings: The progress of this project has been hampered by the lack of an adequate supply of healthy, pure bred strain A female mice of the Heston sub-line. Most of the results can be considered as only preliminary in nature because of the small number of animals used in the experiments. However, these pilot experiments have yielded enough information to guide the future

(Use reverse and additional pages, if necessary)

course of the project. Included under this project is a study of the efficiency of two tetrazolium salts as hydrogen acceptors. This study has been completed. The results of this project can be summarized as follows:

A. Effect of feeding Purina Laboratory Chow on liver tumor production: Since earlier work showed that a high content of riboflavin in the semi-synthetic diet did not protect the mouse against liver tumor formation by o-aminoazotoluene (OAT), this experiment was set up to test whether a semi-synthetic diet (low protein, high carbohydrate) was itself necessary for liver tumor production. OAT dissolved in corn oil was incorporated into finely ground Purina Chow pellets and fed to mice for periods of 60 and 120 days. All mice were killed after 120 days. 33 percent of the mice fed the dye for 60 days developed tumors while 83 percent of the mice fed the dye for 120 days developed tumors. The results indicate that a semi-synthetic diet is necessary for rapid tumor production, since 80 percent to 100 percent of the mice fed the dye in such a diet for 60 days developed liver tumors.

B. Production of tumors in BALB/c mice: Under conditions which resulted in the production of liver tumors in 80 percent to 100 percent of strain A female mice, liver tumors appeared in only 18 percent to 25 percent of BALB/c mice. This confirms the earlier finding of Andervont that BALB/c mice are less susceptible to the carcinogenic action of OAT than A mice.

C. Effect of stilbestrol on liver tumor production by OAT in strain A female mice: This preliminary experiment with small groups of mice shows a positive correlation between the length of time a pellet of 10 percent stilbestrol in cholesterol remains in strain A female mice and the percentage of liver tumors produced in these mice by feeding OAT. This experiment is being repeated with larger groups of animals.

D. Comparison of the reduction of two tetrazolium salts with the succinoxidase activity of mouse tissues: A method for measuring the succinic dehydrogenase system in homogenates of mouse liver, kidney, and brain by the reduction of neo-tetrazolium chloride (NT) and 2-(p-iodophenyl)-3-(p-nitrophenyl) -5-phenyl tetrazolium chloride (INT) was compared with the manometric method of Schneider and Potter for measuring the succinoxidase system. It was found that the ratio of oxygen consumption of liver, kidney, and brain was 1:1.75:0.32 and the ratio of the optical density of the NT formazan produced by these tissues was 1:1.65:0.33. This indicates that, on a

relative basis, the tetrazolium method measures the succinic dehydrogenase system with the same adequacy as the manometric method. In addition, a given amount of liver homogenate in a given time interval will take up 2.45 micromoles of oxygen, and will reduce INT and NT to produce 1.28 micromoles and 0.074 micromoles of formazan, respectively. Thus, INT is one-quarter as efficient as molecular oxygen, but is roughly ten times more efficient than NT as an electron acceptor in this system.

This method will permit accurate estimate of succinic dehydrogenase activity to be made on very small, developing tumors.

Significance to the program of the Institute: In order to gain a more fundamental knowledge of the cause of cancer, more information is needed concerning the biochemical and morphological events which occur in the cell that is undergoing the malignant transformation. This project is composed of a series of studies with a biological system wherein it is possible to investigate the process of carcinogenesis in one type of cell, the hepatic parenchymal cell.

Proposed course: A quantitative cytological and histochemical study of the early stages of tumor formation in these animals is planned.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 409
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$5,360	$1,770	$7,130	1/3	1/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No items for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 410
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cellular Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Transparent Chamber and Diffusion Chamber Research
PROJECT TITLE

7. Glenn H. Algire
PRINCIPAL INVESTIGATOR

8. Doctors R. M. Merwin and Virginia J. Evans
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

RESEARCH PROGRAM IN TRANSPARENT AND DIFFUSION CHAMBER PROJECT.

Problem No. 1. Developments in Diffusion Chamber Methods.

Objectives:

In previous work porous membranes have been sealed to plastic rings to form diffusion chambers for the cultivation of cells in vivo. In certain problems it would be desirable to use more isoporous membranes of the Gradacol type, which are furnished as wet filters, and thus cannot be sealed to plastic rings.

(Cont.)

(Use reverse and additional pages, if necessary)

A second aim has been to obtain filtrates from diffusion chambers for chemical or serologic analysis.

Methods employed:

A new type of ring has been designed with beveled surfaces, so that the filters may be pressed together between the surfaces of contact of the plastic rings, thus avoiding the need for adhesives as a primary seal; diffusion chambers having a capacity of 0.75 ml. were designed to obtain filtrates for analysis.

Major findings:

These improvements have made it possible to use filters of carefully calibrated porosity down to 4 millimicrons; sampling chambers have been used in studies of passage of antibodies and other proteins.

Significance for Cancer Research:

These advances in techniques are of importance in problems of the invasiveness of cancer cells, of cell nutrition, in survival of heterografts, and of the transmission of leukemia.

Proposed course:

The modified diffusion chambers have been used in the above problems.

Problem No. 2. Factors affecting the survival of heterografts in diffusion chambers (with Doctor Virginia Evans).

Objectives:

A study of human cells in diffusion chambers in mice in relation to induction and response to immunity. To determine whether filters of suitable porosity would permit cellular proliferation but block the induction of cytotoxic antibodies by heterografts.

Methods:

Human cells (HeLa strain) and skin epithelia from tissue culture were placed in duffision chambers using filters of graded porosity. Observations were made both in vivo and as histologic preparations after intraperitoneal implantation and removal at intervals thereafter for fixation.

Observations were made also on several human tumors obtained at surgery or postmortem.

Major Findings:

(1) Human cells (HeLa and skin from tissue culture) grew progressively in diffusion chambers without signs of injury for approximately 10 days.

(2) Cellular degeneration occurring by 15 days in type HA (.450 micron average porosity) was found to be due to cytotoxic antibodies induced in the host by HeLa cells within the chambers.

(3) It is not yet clear whether immunity was induced by cells which escaped or by non-cellular antigenic material.

(4) Cellular degeneration was delayed beyond 15 days in chambers using filters of 10 millimicron average porosity, and with a small initial inoculum. Further work is in progress.

(5) Several fresh human tissues did not show degenerative changes by 15 days. Further work is in progress.

(6) Diffusion chambers provide a means for studies of the response of human or other heterograft cells to various experimental procedures for up to 10 days before immune reactions cause degenerative changes.

Significance for cancer research:

This is a study of the growth of human tissues in untreated laboratory animals. Possible application to diagnostic and chemotherapeutic problems of cancer are being investigated.

Proposed course:

Further work will be done using fresh human tissues, such as blood or tissues from leukemic patients.

Studies will be continued on the effect of porosity of filters and of size of inoculum on the induction of immunity to heterografts, with the aim of blocking the immune reaction.

Problem No. 3. Studies in carcinogenesis using diffusion chamber methods.

Objective:

The diffusion chamber method for "tissue culture in vivo" provides a new approach to certain problems of chemical carcinogenesis. Earle and associates have found that normal adult fibroblasts grown in tissue culture in a completely heterologous media in the absence of deliberately added MCA give rise to sarcomas when inoculated into mice. One possible explanation for these results has been "growth in the heterologous media." This explanation would be ruled out if normal or embryonic mouse tissue underwent a malignant change when enclosed within diffusion chambers.

Goldblatt, following experiments and theories of Warburg (1927), has presented evidence that normal fibroblasts in tissue culture undergo a malignant change if subjected to conditions of intermittent anoxia. Through selecting filters of increasing thickness, and of consequent greater diffusion distance, one could study the effect of this variable on neoplastic change.

The immediate objective is to ascertain whether normal adult or embryonic subcutaneous tissue enclosed within diffusion chambers undergo neoplastic transformation.

Methods employed:

1. To determine whether the materials (plexiglasII, and Millipore filters) are carcinogenic for mice - (by intraperitoneal and subcutaneous implantation).

2. Muscle fragments from young mice (strain C3H) were enclosed within diffusion chambers and placed intraperitoneally into adult hosts of the same strain. After 12 months these were reimplanted (open) into new hosts which had received 400-425 r. total body irradiation.

Major Findings:

(1) Normal connective tissue cells survive in diffusion chambers for at least 12 months.

(2) Reimplantation of these cells into irradiated hosts after 12 months has not led to the production of tumors.

(3) In experiments lasting 1 year no sarcomas were produced from implantation of materials used in diffusion chambers (cellulose esters and plexiglas).

Significance for Cancer Research:

Increased understanding of factors involved in the transformation of normal to neoplastic cells is of fundamental importance in cancer research.

Proposed course of problem:

This experiment will be repeated, using larger chambers - since size of inoculum has been shown by Sanford to be important in the percentage of "takes"; homologous cells will be used so that any tumors which arise may be distinguished by genetic tests from tumors which may have arisen from host cells.

Experiments will be undertaken using mouse embryonic tissues.

In cooperation with Doctors Brackett and Olsen it is planned to measure oxygen in solution in filtrates entering through filters of graded porosity.

Problem 4. Invasiveness of leukemia.

Objectives:

Mouse leukemia cells differ greatly in their capacity to invade the tissues of the host. Studies were undertaken to determine whether this property is correlated with the ability of these cells to pass through filters of graded porosity.

Methods:

Fragments of leukemia 1210 were placed in diffusion chambers using filters of from 10 millimicrons to 0.450 microns average porosity. Biological and histologic studies were made to determine the fate of the cells. Double layer filters were used to distinguish between escape of cells and alternative possibilities.

Major findings:

Leukemia 1210 has consistently escaped from these chambers. Since only 4 cells, according to Doctor Law, are required for 100 percent "takes" of this tumor, it

appears that occasional pores exist which are much larger than the manufacturer's estimates of pore size and pore variation. Methods of construction and assembly of diffusion chambers are also being studied in this connection.

Significance for Cancer Research:

These studies are concerned with factors in the spread of cancer cells in normal tissues. One must consider, in this connection, not only the porosity of the filters and the range of variation in pore size, but also the number of cells required for a "take", and the migratory properties of the leukemic cells.

Proposed course:

(1) To explore these problems using presumably more uniform filters (Gradacol).

(2) To study other available mouse leukemias.

Problem No. 5. Vascularization of grafts of normal and neoplastic tissues. (The major portion of this work is being done by Doctor Ruth Merwin. Details will be found in her report).

Objectives:

To study by microscopic observation in living animals the early stages in the establishment of a blood supply between graft and host. To determine the role of the host and of the graft in this process in normal and neoplastic tissues.

Methods:

Observations are made with the microscope of early stages of vascularization of grafts which were placed near superficial blood vessels in nerves so that details of vascularization could be observed.

Major Findings:

(1) Endothelium in certain normal embryonic tissues grows out from the graft to establish connections with the host. The endothelium of the host is not stimulated to proliferate by the presence of grafts of normal tissue.

(2) Rapidly growing tumor grafts elicit a vigorous proliferation of new capillaries from the adjacent host

capillaries; endothelium in tumor grafts exhibits little or no activity.

Significance for Cancer Research:

Continued growth of tumors depends upon their establishment and maintenance of a good blood supply. This work serves as a base-line for future studies on chemical factors in endothelial proliferation.

Proposed Course:

(1) To compare various types of tumor tissue with respect to their capacity to elicit endothelial proliferation.

(2) To investigate chemical factors which may influence endothelial growth.

Problem No. 6. Diffusion chamber studies of cellular immunity to homografts.

Objectives:

In previous reports diffusion chamber methods were described for obtaining growth of homografts in genetically incompatible host animals. Current work with diffusion chambers has been directed toward the following questions:

1. For how long do homografts survive?

2. Do homografts in diffusion chambers immunize the host?

3. What is the chemical composition of filtrates obtained from the chambers?

4. Do antibodies pass through the filters in vivo?

Methods:

Diffusion chambers were used in which tissue grafts grow as a "tissue culture in vivo" without direct contact with host tissues; tests for host immunity were done using unvascularized Harderian gland as an indicator; special chambers were used for the collection of filtrates.

Major Findings:

(1) A mouse mammary adenocarcinoma survived for at

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PUBLIC HEALTH SERVICE -NATIONAL INSTITUTES
OF HEALTH INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 410
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Merwin, R. M. and Algire, G. H.: The role of graft and host vessels in the vascularization of grafts of normal and neoplastic tissue. J. Nat. Cancer Inst. 17: 23-33, 1956.

Algire, G. H., Weaver, J. M. and Prehn, R. T.: Studies on tissue homotransplantation in mice using diffusion chamber methods. Annals N. Y. Acad. Sci. (in press).

Algire, G. H.: In vivo cell culture. Univ. of Michigan Medical Bulletin (in press).

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Feb. 1. State Univ. of New York, Brooklyn, N. Y., Dept. of Anatomy: Studies of host-graft interaction in transplantation, with special reference to survival of homografts in diffusion chambers.

Feb. 20. Naval Medical Research Institute, Bethesda, Md.: Studies of host-graft interaction in transplantation, with special reference to survival of homografts in diffusion chambers.

May 11. Research Conference. N.I.A.I.D.: Discussion of "The Immunology of Homotransplantation.

June 18-28. Biology Council, The National Academy of Sciences, National Research Council. Invited by Doctor Paul Weiss to participate in a Workshop in Developmental Biology, Bar Harbor, Maine.

August 2. Lecture "In vivo cell culture" for The Course in Principles and Methods of Tissue Culture". Univ. of Colorado School of Medicine, Denver, Colorado.

(cont.)

Project 410 (cont.)

17. Sept. 14. Third University of Michigan Cancer Retreat. Lecture "Microscopic studies of living mice by transparent chamber methods; host-graft interactions in transplantation of normal and neoplastic tissues.

Oct. 2. Invited by Doctor Paul Weiss to participate in a Work Conference on Biological Foundations of Wound Healing and Tissue Repair. Rockefeller Institute for Medical Research, New York City.

Oct. 7. Discussion of paper by Doctor George Klein "Cancer studies in fields collateral to tissue culture" at Decennial Review Conference on Tissue Culture, Woodstock, Vermont.

Dec. 12. Washington Academy of Surgery. Transparent Chamber Studies of Host-graft Interaction.

Miscellaneous:

Interest was shown by other investigators in transparent and diffusion chamber methods. Demonstrations and instructions were given to approximately 30 workers who visited the laboratory for variable periods of time.

Dr. Robert Duthie has come as a guest worker from the Dept. of Surgery, University of Edinburgh, to remain 6 months for instruction in methods developed in this laboratory.

Our motion pictures "In vivo cell culture and "Diffusion Chamber Methods" have been used in the lectures listed above and have been loaned to:

Dr. George Snell, Roscoe B. Jackson Laboratory, Bar Harbor, Maine;

Dr. Still, George Washington Univ., Dept of Physiology;

Dr. John M. Converse, Dept. of Plastic Surgery, Cornell Univ. School of Medicine;

Dr. Jacob Fine, Dept. of Surgery, Beth Israel Hospital, Boston, Mass.

Dr. Edward D. Churchill, Dept. of Surgery, Massachusetts General Hospital, Boston, Mass.

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 410
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Inductive interaction in the development of the mouse metanephros. J. Exp. Zool. 130: 319-340, 1955

(2) Transfilter induction of tubules in mouse metanephrogenic mesenchyme. Exper. Cell Res. 10: 424-440, 1956

(3) Inductive tissue interaction in development. In press. Advances in Cancer Research, Vol. IV.

(4) Kidney tubule induction in mouse metanephrogenic mesenchyme without cytoplasmic contact. (With A. J. Dalton) J. Exp. Zool. In press.

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 414
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cellular Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Transparent chamber research
PROJECT TITLE

7. Ruth M. Merwin
PRINCIPAL INVESTIGATOR

8. Dr. C. C. Congdon, Dr. K. K. Sanford, and Dr. G. H. Algire
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Problem 1: To study the repopulation of the hematopoietic tissues of x-irradiated mice after the intravenous injection of normal homologous marrow.

Objective: A number of recent studies and this one, have shown that homologous cells injected intravenously into irradiated animals contribute to the repopulation of the blood, marrow, spleen, lymph nodes, and thymus. In continuing this work, we wished to make the study more quantitative.

(Use reverse and additional pages, if necessary)

Method: Mice of one inbred strain were irradiated with 900 r and each was protected from this otherwise lethal dose by an intravenous injection of homologous marrow. At various intervals thereafter, the hematopoietic tissues of the protected mice were tested to see if homologous cells were present. The tissues to be tested were injected into test mice of the same strain as the irradiated mice. Each of the test mice carried a nonvascularized homograft of harderian gland tissue. Nonvascularized homografts do not initiate immunity in the host, but if the injected tissues being tested contained homologous cells, immunity was initiated in the host and caused the graft to disintegrate. Quantitative estimates were made by determining how small an amount of tissue from the protected mice would cause the test mouse to develop immunity.

Major findings: Studies on the amount of homologous tissue present in marrow, thymus and lymph nodes allow one to estimate that at least 1/4 and possibly almost all of the cells of these tissues were homologous at 60 to 100 days after protection.

Significance: It is important to understand the mechanism by which the injection of homologous marrow protects animals that have received whole body x-irradiation.

Proposed course: This work is being prepared for publication. Some preliminary work is being done with Dr. Willie Smith to find out whether injections of blood from mice carrying the A280 tumor protect because the introduced cells repopulate the hematopoietic tissues of the irradiated mouse.

Problem 2: Tumor growth and vascularization. Initial vascularization of transplants of tumor and normal tissue. Ruth Merwin and Glenn Algire.

Objective: The object of this study is to evaluate the significance of the activity of the graft and host vessels in the initial vascularization of grafts.

Methods employed: Microscopic observations were made on grafts placed in transparent chambers in living mice. With the usual methods of studying grafts one must focus through the graft in order to see the area in which the graft and host vessels establish connections. Therefore, grafts were placed so the area in which these changes take place would be beside rather than under the graft.

Major findings: The surviving vessels in grafts of normal tissues produce sprouts which may be active in establishing connections between the vessels of the graft and those of the host. On the other hand, the vessels in grafts of tumor tissue survive only in some grafts and do not produce new vessels. Grafts of normal tissue of the size used did not cause host vessels to form new sprouts, whereas grafts of a similar size of a mammary adenocarcinoma caused a rich network of new sprouts to form.

Significance: This is a problem concerned with the relationship of tumor cells to the surrounding normal tissue.

Proposed course: The work on this subject has been published. A comparative study of the effect of implants of different types of tumors on host vessels is being considered.

Problem 3: To study the response of the host to implants of cells of malignant or nonmalignant strains. Ruth Merwin and K. K. Sanford.

Object: To see if the growth capacity or metabolic characteristics of such cell strains could be correlated with changes in the host tissues surrounding grafts of these tissues.

Methods: The tissue to be tested was placed subcutaneously in a mouse in a transparent chamber. The reaction of the host to these implants was observed, especially the changes in blood vessels, such as hyperemia and the production of new sprouts.

Major findings: None as yet.

Significance: It is important to understand the interrelationships between tumor cells and the surrounding tissues.

Proposed course: To continue testing various tissues to find correlations between host reactions and tissue characteristics.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 414
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$8,040	$2,640	$10,680	1	0	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	0	1	0

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 414
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Merwin, R. M. and Algire, G. H.: The role of graft and host vessels in the vascularization of grafts of normal and neoplastic tissue. J. Nat. Cancer Inst. 17: 23-33, 1956.

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 416
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cellular Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The mechanism of homograft destruction.
PROJECT TITLE

7. James M. Weaver
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Previous work using the diffusion chamber technique indicated that homografts were destroyed by host cells, probably lymphocytes, rather than by circulating antibodies. However, in such work only fixed cells could be studied, and it became obvious that it was most desirable to study the possible interaction between living "immunized" lymphocytes and the appropriate "target cells" in vitro. It now appears that such a method has finally been developed. This followed an in vivo study of homografted ascites tumor cells which could be withdrawn previously from the peritoneal cavity in a state suitable for cytological analysis of the mechanism of their destruction. These tumor cells died with the lymphocytes of the host intimately attached to their surfaces. Other types of host cells

were not seen to play any role in their death. The next procedure was to combine tumor cells with immunized peritoneal lymphocytes in vitro. Here they were destroyed in a similar way. Preliminary evidence indicates that the donors of these immunized lymphocytes subsequently carried circulating antibodies against the tumor cells used for immunization.

Homografted Ascites Tumor Cells Studied in Vivo.

The ascites tumor cells used were derived from a dba/2 thymoma. An almost pure population of these cells could be obtained 10 to 14 days after inoculation. In particular, very few or no lymphocytes could be found in these cell suspensions. Because of the distinctive morphology of these tumor cells, it was possible to distinguish them from normal peritoneal cells which consisted mainly of lymphocytes and macrophages with a few eosinophiles and mast cells.

Inoculation of 0.1 ml. (which contained approximately 1,500,000 cells) of this ascites tumor fluid did not produce ascites or kill C57BL/6 mice, although it was usual for such an inoculation to kill 100 percent of dba/2 mice in about 16 days. Following the intraperitoneal injection of these cells into C57BL mice, they could be found (some even containing mitotic figures) in diminishing numbers for 4 to 6 days, and after a week they had disappeared. However, when injections of tumor cells were repeated a second and a third time, 7 and 10 days respectively after the first inoculation, these tumor cells died within the peritoneal cavity in a few hours, and there was strong evidence that this was caused by the peritoneal lymphocytes. This evidence will be described below because similar results were obtained during the in vitro procedures.

During the tumor cell destruction in vivo, plasma cells could not have been involved because these cells were not observed in the peritoneal fluid of mice. Similarly, neutrophiles were not considered of importance because they were present in very small numbers until after most of the tumor cells had died, when there was a marked increase in their numbers. Macrophages were seen only to engulf dead tumor cells - never viable cells.

Tumor Cells Combined with Peritoneal Lymphocytes in Vitro.

After the C57BL/6 mice had been immunized by three injections of dba/2 tumor cells, as described above, peritoneal fluid again containing predominately lymphocytes and macrophages was withdrawn 11 to 14 days after the initial injection. This was combined with fresh tumor cells in depression slides at room temperature. About one-third of the C57BL/6 lymphocytes which chanced to come into contact with tumor cells for a period of a few minutes became adherent to them. These contacts were the result of active or passive movement on the part of the lymphocytes, the tumor cells, or both. So far, there is no evidence that the lymphocytes were attracted to the tumor cells from a distance by chemotaxis. Usually, one lymphocyte became attached to one tumor cell, but occasionally a lymphocyte was seen to be adherent to two tumor cells or, conversely, sometimes more than one lymphocyte adhered to a tumor cell. The intimacy and the duration of this attachment varied considerably. Sometimes the lymphocytes would adhere weakly for a short time and then move away, or more frequently they would adhere tightly and push the tumor cells for considerable distances, often indenting or circling them.

About half of the tumor cells with attached lymphocytes died in from 10 to 90 minutes - the average time was about 60 minutes. The tumor cells without attached lymphocytes lived for at least 4 hours - as did the other cells in the preparation. The lymphocytes which killed tumor cells did not die. (This is to be contrasted with previous diffusion chamber results where they did die, and this apparent discrepancy remains to be explained.)

In similar control cell combinations of tumor cells with peritoneal cells from normal C57BL/6 mice, the phenomenon of lymphocytic adherence was never observed and all of the cells lived for at least 4 hours. Normally, in contrast to macrophages, lymphocytes are not phagocytic or particularly adhesive.

No difference was noted between the behavior of macrophages from normal or previously immunized C57BL/6 mice toward the tumor cells.

The mechanism whereby the immunized lymphocytes killed the tumor cells is unknown although they may have carried antibodies to these cells. If such a lymphocyte contained a sufficiently large number of antibody molecules, presumably it would have had this lethal effect. At any rate, the above

evidence certainly suggests that the lymphocytes of an immunized mouse are heterogenous in nature because some are inactive toward tumor cells, whereas others adhere without killing, and others adhere and kill rapidly.

It is certain that the active lymphocytes did not kill by phagocytosis. This was never observed and frequently the tumor cell victim was much larger than the lymphocyte which attacked it. The two cells of a tumor cell-lymphocyte pair retained their individuality although often they were so tightly adherent that cell surfaces were difficult to distinguish. Further, no exchange of material between the two cells was ever observed.

The type of degeneration of the tumor cells with attached lymphocytes varied, usually beginning in the cytoplasm, perhaps at the cell surface, with the nucleus becoming involved later. The attached lymphocytes often maintained contact with these cells long after the latter were dead. Immunized lymphocytes were also observed to adhere to tumor cells initially dead, e.g., to those taken from moribund dba/2 donors. This suggests that the dba/2 specific antigen involved is possibly subcellular in nature.

No morphologic difference was observed between the lymphocytes of a normal C57BL mouse and those from an immunized mouse, nor was it possible thus to distinguish between the "active" and the "inactive" lymphocytes of the immunized mouse. Similarly, it has not been possible to correlate the degree of activity of lymphocytes with their size.

Because the "active" lymphocytes of an immunized mouse do not lose this property on washing, there is nothing present in the cell-free peritoneal fluid of these animals which is essential for these cells to kill tumor cells.

Recently a quantitative type of in vitro technique of combining cells has been used, although with this method there is some loss of cytological detail. Large numbers of tumor cells were mixed in tubes with peritoneal cells from normal C57BL/6 or with those from previously immunized C57BL/6 mice. The tubes were centrifuged to bring the cells in contact and then the cells were resuspended. Over a period of two or three hours, samples were then removed for microscopic examination or for counts of the number of tumor cells in a hemocytometer. The results of microscopic examination were quite similar to those originally obtained by combining such cells on a slide. The counting in a hemocytometer demonstrated that it was possible to study millions of tumor cells, rather than only

the dozen or more which could be studied on a slide. A large fraction of these tumor cells in the tubes combined with the immunized C57BL lymphocytes were destroyed before there was a significant decline in the number of tumor cells in control tubes combined with normal C57BL lymphocytes.

Significance of Results.

It is hoped that this work will be of value in determining the mechanism of homograft immunity which has, until recently, been most confused. Possibly such knowledge could be useful in analyzing the mechanisms of tumor progression because there has been increasing evidence that primary tumors as well as transplanted tumors may be antigenic to their hosts. The rare spontaneous cancer cures and the not-so-rare erratic progression of primary tumors and their metastases may be manifestations of such antigenicity and of the hosts' attempts to respond to it. Accordingly knowledge of the mechanism of homograft immunity, an immunity presumably more closely related to tumor auto-immunization than other types of immunity, is important.

Proposed Course.

1. Little more qualitative data are needed except that time-lapse motion pictures will be used to record the destruction of tumor cells by immunized lymphocytes. This will enable us to be more certain that a chemotactic attraction of the tumor cells which may be present for the lymphocytes has not been overlooked and that there is indeed no passage of visible material from the lymphocytes into the tumor cells.

2. More quantitative data (cell combinations in tubes followed by cell counts) will be obtained but this would appear to be merely a matter of repeating on a larger scale procedures already developed.

3. One final and probably very important matter will be investigated further because its omission could lead to confusion if the experiments described above were repeated in a slightly different manner by others. Actually this might lead to results which could seem to invalidate the above findings.

This matter to be investigated further results from preliminary data which indicate that, if C57BL/6 mice are immunized as described above (on days 0,7, and 10) and are then reinjected with tumor cells more than 16 days after the first inoculation, these latter tumor cells are again destroyed very rapidly, but lymphocytes (or other host cells) do not appear to be involved under these conditions. The tumor cells simply degenerate and die without lymphocytes adhering to them. These data

need to be confirmed. It is suspected that tumor cell death under these circumstances is the result of circulating antibodies. If so, probably they could be demonstrated in vitro using the sera of such animals. This experiment will be performed.

If, as anticipated, circulating antibodies are demonstrated under these conditions, there might appear to be an incompatibility between these results and the lymphocytic destruction already described as well as with our earlier diffusion chamber results. However, this need not be so. It could be that animals homografted once do not bear circulating antibodies, but that their lymphocytes do; whereas animals homografted more than once initially have lymphocyte transported antibodies and later cell-free circulating antibodies. It should also be borne in mind that an animal homografted once with a tumor which grows for a considerable time before regression could be equivalent to an animal repeatedly grafted with a less vigorous tumor.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 416
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$8,040	$2,640	$10,680	1	0	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	0	1	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No items for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 417
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. General Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Investigation of the role of genes and their relationship to non-genetic factors in the development of cancer.
PROJECT TITLE

√7. W. E. Heston
PRINCIPAL INVESTIGATOR

8. Margaret K. Deringer, and George Vlahakis
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine the role of genes in the development of tumors in mice, to estimate the number of genes involved and to locate them on specific chromosomes, to determine the physiologic pathways through which the action of the genes becomes manifest in increasing or decreasing the occurrence of tumors, to test various agents, particularly mutagens, for carcinogenic action and to relate their action to that of the genes in the production of tumors.

Methods employed: We maintain in our laboratory the inbred strains of mice most suitable for research on pulmonary tumors and mammary gland tumors. These include: 1) Strain A, the strain most

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highly susceptible to pulmonary tumors with an incidence of 90 percent in animals 18 months of age, 2) Strain C57L, the strain most resistant to pulmonary tumors, only 3 such tumors having been observed in this strain throughout its history, 3) Strain C3H, the strain most highly susceptible to mammary tumors with an incidence of over 95 percent in breeding females at an average age of less than 8 months, and 4) Strain C57BL in our line of which no mammary tumors have been observed. In addition we have developed strain $C3H_f$ which is genetically comparable with C3H but does not have the mammary tumor agent, and we have several strains carrying specific genes for linkage studies.

Appropriate crosses of these strains are made for the study of the segregation of specific genes and their effects on tumor development. Carcinogens are injected into these specific hybrids for studying the relationship of the specific genes to the action of the carcinogen. The route of injection will vary with the carcinogen used, the hydrocarbons usually being injected intravenously as a colloidal dispersion, and urethan intraperitoneally in solution and the mustards intravenously in solution.

The induction of pulmonary tumors in strain A mice is considered the most delicate test for the carcinogenic action of an agent. Response is measured by counting the tumors at a specific time following the injection. This gives a quantative measure of response and yields data to which statistical tests can be readily applied. Counting of the nodules is done under the dissecting microscope; and in the case of the oxygen work, the lung was fixed by intratracheal injection of the fixative prior to removal from the chest cavity. This has improved the accuracy of the count.

All tumors are routinely sectioned and stained for histologic examination.

Aside from appropriate genetic crosses in the mammary tumor work, foster nursing of susceptible young is used for testing for the presence or absence of the agent. The strain $C3H_f$ from which the agent was removed by foster-nursing has been used extensively for this purpose, but more recently we have used strain $C3H_e$ developed by Dr. Deringer from fertilized ova transferred from strain C3H to C57BL. Pedigree charts are kept and it is through inspection of the occurrence of females with tumors in them that we obtain the best proof for the presence or absence of the agent.

Major findings: During the year considerably more data on the studies of linkages between various genes and spontaneous pulmonary tumors were obtained. We now have a total of seven genes located on five different chromosomes that have been shown to be related to the development of pulmonary tumors. The tumors have been spontaneous or those induced by a number of agents including methyl cholanthrene, dibenzanthracene, urethan, nitrogen mustard. Each of the seven genes has shown linkage regardless of whether the tumors were spontaneous or induced by any of the different agents except for the two genes, lethal yellow on chromosome V and flexed tail on chromosome XIV. These two genes were linked with the spontaneous tumors and those induced by each of the agents except dibenzanthracene. These tests have been repeated with large numbers of animals, and apparently the observation is real. A different mechanism for dibenzanthracene is, therefore, indicated even though it is difficult to understand why there should be a different mechanism for the two closely related compounds, dibenzanthracene and methyl cholanthrene, while there is no such difference between methyl cholanthrene and the unrelated compounds, urethan and nitrogen mustard. The reason that only these two genes showed this irregularity may be related to the fact that the "linkage" with them is due to the effect of these two genes themselves on pulmonary tumors, whereas in the case of the other five genes true linkage, i.e., a tumor gene located on the same chromosome with the gene tested, may be represented. These results were presented at the International Genetics Symposium in Tokyo in September.

The preliminary study of the effect of high concentration of oxygen on the induction of pulmonary tumors was completed and published during the year. This study was undertaken because of our interest in relationships between carcinogenesis and mutagenesis and because mutation rate in lower organisms has been shown to increase with increase in concentration of oxygen. Melanotic tumors in Drosophila larvae have also been increased with exposure to increased oxygen. In our study done in collaboration with Dr. A. W. Pratt, strain A mice injected with .1 mg dibenzanthracene and immediately thereafter exposed to approximately 100 percent oxygen for 48 hours developed on the average approximately twice as many pulmonary tumors as those injected with .1 mg dibenzanthracene and kept in air.

More data have been gathered in the dose response studies of induction of pulmonary tumors in strain A mice. These studies were undertaken to determine whether the results would fit a "one hit" hypothesis such as the tumor

being the result of a dominant mutation in the cell, a "two-hit" hypothesis such as the tumor resulting from a recessive mutation, or some other mechanism. Preliminary published results showed a straight line response measured by average number of tumors induced with doses of dibenzanthracene ranging between .1 mg and .5 mg. Extension of this straight line, however, fell below the observed average number at zero dose; in fact it fell below zero number of tumors. The response curve was later extended as a straight line to dose .01 mg but was found to deviate upward at doses of .005 and .0025 mg but at these two doses not 100 percent of the animals had tumors. Thus, between doses .01 mg and .5 mg the response curve is compatable with a "one-hit" hypothesis.

Since the dibenzanthracene was given as a colloidal dispersion, it was considered that what we were actually studying might be merely distribution of particles with each cell that was exposed receiving a maximum concentration rather than that we were studying response to different concentrations of the carcinogen. A dose response curve was, therefore, determined for urethan to which being in solution the objection in respect to the particles could not be raised. Points were obtained for the controls injected with water and for doses of urethan ranging from 1.25 mg to 40 mg each given in a single injection. Although statistical analyses of these data are not complete, it is apparent that these points, even including the one at zero dose, do not deviate from a straight line. Thus, these results appear to be compatible with a "one-hit" hypothesis and are what one could expect if the tumors resulted from a dominant mutation.

Since it seemed desirable to establish a dose response curve for a strain genetically more resistant to pulmonary tumors than strain A, strain $C3H_f$ mice were injected with urethan in doses ranging from 1.25 mg to 40 mg. The response for each dose was either not significantly different from that of zero dose, or was very low, and it could not be determined what kind of a curve, if any, the points fit. However, there was an indication that the incidence of hepatomas increased with an increase in dosage of urethan. This was a new observation that required repeating. We now have data confirming the observation that injection of single doses of urethan in strain $C3H_f$ mice will increase the incidence of hepatomas. The sex difference noted with spontaneous hepatomas also was found when urethan was injected.

This study was extended to multiple injections of 20 mg each of urethan in $C3H_f$ mice and also in C3H and $C3H_e$ mice. Eight injections of 20 mg each did not increase the incidence of hepatomas in $C3H_f$ mice over that observed following one injection. Furthermore, in the C3H and $C3H_e$ mice, the incidence of hepatomas following the 8 injections of urethan was not significantly greater than the incidence in the controls. However, the incidences in these C3H and $C3H_e$ mice were abnormally high compared with observations on breeding mice of these strains and significantly higher than that in the $C3H_f$ controls. Thus, further data should be obtained to clarify some of these points before publication.

The number of pulmonary tumors in the animals receiving 8 injections of urethan of 20 mg each was greater than that in those receiving one injection of 20 mg. Furthermore, more pulmonary tumors occurred in the C3H mice than in the $C3H_f$ or $C3H_e$ mice, but the difference was not great. Whether this difference was due to the fact that the C3H mice have the milk agent whereas the $C3H_f$ and $C3H_e$ do not, or was due to a genetic difference, or to some other difference, was not evident.

Studies on the action of the lethal yellow gene in increasing pulmonary tumors in which the brown litter mates of the yellow mice are made fat like the yellow by injecting them with gold thioglucose and the weight of the yellow mice is held down comparable to that of the browns by starvation are in progress with no final results for reporting.

The studies on mammary tumors in mice continue to be chiefly concerned with the mammary gland tumors in females without the agent and with the genic control of the propagation and transmission of the agent. The $C3H_f$ line is now in the 30th and 31st generation of brother x sister inbreeding since being deprived of the agent by foster-nursing, and females through the 26th generation have died at advanced age. The mammary tumors continue to appear in from 20 to 30 percent of the breeding females with the females with tumors scattered throughout the pedigree chart rather than segregating out as high-tumor sublines. This is probably the best evidence that we have that the mammary tumor agent is not involved in the development of these tumors. We have discontinued the breeding of our second agent-free subline $C3H_f/2$ and our colony of the Andervont agent-free subline $C3H_f/An$ since mammary tumors were appearing in these lines much the same as in the original $C3H_f$ line.

During the year the extensive study of the genetic control of the mammary tumor agent was published. Briefly, the major findings of this study were as follows: 1) the agent could be eliminated from a line by backcrossing to the resistant strain through three generations indicating that the propagation and transmission of the agent were controlled by relatively few genes and possibly only one, 2) there was no evidence that the agent could arise de novo, 3) in genetically susceptible mice without the agent some mammary tumors arose at an advanced age, 4) occasionally the agent was transmitted from the males with the agent to the susceptible females at the time of mating and from the resulting litters high-tumor sublines arose, 5) greater histologic variation was found in the mammary tumors that arose in the older females in the absence of the agent than in those that arose in the younger females with the agent. A large number of tumors other than of the mammary gland, some of which were of unusual type, were observed in these old females without the agent. Detailed histologic descriptions of these are being prepared by Dr. Thelma Dunn for future publications. One paper on the subcutaneous fibrosarcomas will appear in the November 1956 issue of the J.N.C.I.

Significance to program of Institute: This program is directed toward the eventual control of cancer in man through a thorough understanding of the etiologic factors involved. Basic among these factors are the genes which in conjunction with various non-genetic factors that may be introduced, eventually result in the occurrence of the cancer. We have pregressed further in the strictly genetic analysis of lung tumors in the mouse, in the identification of specific genes involved, and in the relationship of the genes to the various carcinogenic agents than has been accomplished in respect to any other tumor either in experimental animals or man. The mammary tumor in the mouse is particularly interesting in that there are involved other intrinsic factors besides the genes including the hormonal agents and the mammary tumor agent. Our results are significant in the general problem of the genetic control over a virus. It is also of significance that the disease, in this case the tumor, can appear without the virus. In general, these findings on the mouse should be of significance in directing our thinking of the problem in man and in outlining investigations on the etiology of cancer in man.

Of specific interest was the observation of increased pulmonary tumors associated with increased concentration of oxygen. These studies are being extended to the possible

effect of exposure of new born mice to high concentration of oxygen on the later occurrence of lung tumors without the introduction of dibenzanthracene. If positive results are obtained here, an investigation into the possibility of such a result from the exposure of new born babies to high concentration of oxygen would be in order. These findings are also of significance in metabolic studies of the induction of cancer, an area in which Dr. Pratt will be particularly interested.

Proposed course of the project: During the coming year the studies on the effects of specific genes on lung tumors will be concerned chiefly with the lethal yellow gene. The project in which the brown mice were made to become obese by injection of gold and the yellow mice were kept at the normal weight level through starvation should be completed. Since, however, this project was set up using dibenzanthracene, and we have since found that the lethal yellow gene has no effect on lung tumors induced with dibenzanthracene, the project may have to be repeated with methyl cholanthrene.

The original experiment on the effect of high concentration of oxygen on increasing induced lung tumors in mice is being repeated with an additional set of controls and with closer measurements of conditions of exposure. The additional controls will also be placed in an exposure chamber under the same conditions as the oxygen exposed mice except that the concentration of oxygen will be that in air. In addition, mice have been exposed at birth to high concentration of oxygen and during the year they will be autopsied and their lung tumors tabulated.

If animals and space are available, the field of relationship between mutagenesis and carcinogenesis will be pursued further through the testing of some additional interesting mutagens for their carcinogenic activity.

During the coming year additional large groups of C3H and $C3H_f$ male mice will be injected with urethan with comparable large groups of control males to attempt to clarify the picture regarding the induction of hepatomas with urethan before publication.

If animal space is available, the difference between strain C3H and strains $C3H_f$ and $C3H_e$ in lung tumors induced with urethan will be studied further through foster-nursing to determine whether or not this difference is due to some agent transferred through the milk.

Future work on the mammary tumors will continue to emphasize the tumors occurring in the absence of the agent. The former publication on the $C3H_f$ strain included females only through the F_5 generation. We now have a large amount of data on females from the F_6 to the F_{26} generation that we hope to get analyzed and published during the year. The analyses should include such items as parent offspring correlation, the number of litters the female gave birth to, the number of the litter in which she was born, classification of the mammary tumors as to histologic type, etc. Other tumors in these females will also be tabulated. An unexplained marked increase in hepatomas through several generations has occurred. An extensive project designed to establish whether or not the genetic control over the mammary tumor agent is due to a single gene is under way. This will be continued through the year although the study will not be completed for another two or three years.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 417
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$48,260	$15,900	$64,160	1	5	6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	5	6	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, and Publications 15. 417
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Heston, W. E., Deringer, M.K. and Dunn, T. B.: Further studies on the relationship between the genotype and the mammary tumor agent in mice. J. Nat. Cancer Inst. 16: 1309-1334, 1956.

(2) Heston, W. E. and Pratt, W. W.: Increase in induced pulmonary tumors in mice associated with exposure to high concentration of oxygen. Proc. Soc. Exper. Bio. and Med. 92: 451-454, 1956.

(3) Heston, W. E.: Effects of genes located on chromosomes III, V, VII, IX, and XIV on the occurrence of pulmonary tumors in the mouse. Proc International Genetics Symposia, Tokyo, Japan. September 6-12, 1956.

(4) Dunn, T. B., Heston, W. E., and Deringer, M. K.: Subcutaneous fibrosarcomas in strains C3H and C57BL female mice and F_1 and backcross hybrids of these strains. J. Nat. Cancer Inst. 17: 639-655, 1956.

(5) Deringer, M.K. and Heston, W.E.: Abnormalities of urogenital system in strain A x C line 9935 rats. Proc. Soc. Exper. Biol. and Med. 91: 312-314, 1956.

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 418
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. General Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Host-Tumor Relationships
PROJECT TITLE

7. Dr. M. K. Barrett
PRINCIPAL INVESTIGATOR

8. M.B. Melroy and M. K. Deringer
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

0. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

In multicellular organisms the morphology and physiology of each cell and each tissue is strongly influenced by other cells or tissues in its environment. There seems to be a cellular ecology which maintains organization and balance from earliest differentiation to death. Ample experimental evidence for such intercellular influences exists.

Cancer may be viewed as a disturbance of such an ecological balance, and from such a view one may derive a definition of cancer. A definition in these terms is the only definition of which I am aware that does not require frequent important qualifications.

(Use reverse and additional pages, if necessary)

For example, some normal tissues have enormous proliferative power and may even be invasive, whereas some malignant cells multiply slowly and may remain localized indefinitely, but in the end the normal usually responds to controlling influences and the malignant does not.

Such a definition is primitive but its constancy is a recommendation. The primitive quality is due to a lack of knowledge of the nature of the forces or influences that modify the intercellular balances. At present we call these influences "immunogenetic" for want of a better term. Our research is directed at learning more about the nature of these influences. The possibility of final practical application of the knowledge is suggested by the occurrence of spontaneous remissions and even regressions in cancer of both man and animals - regressions that appear to be brought about by a resistance on the part of the host.

Methods: The basic procedure is transplantation of standard tumors into hosts of known genetic constitution and studying the effect of experimental variations on the host, the tumor, or both. The disciplinary techniques involved are those of genetics, immunology, and experimental surgery.

Major findings: We previously reported that a host can be made immune to the implantation of a tumor by prior inoculation of washed red cells or by stromata but not when the cells or stromata have been broken up by relatively gentle procedures. We also reported that the antigen was easily destroyed by heat or by formalin. Others have strongly suggested that the antigen concerned is a hemagglutinogen. Available data indicate that, genotypically, these antigens are related, but we believe that phenotypical differences exist and may be important. Accordingly, we have accumulated unpublished data that indicate such differences. This work has been hampered by "histocompatibility" limitations which prevented observation of the 2 types of phenomena in the same mouse. We have made many observations indicating that the "resistance" antigen and the hemagglutinogen are different, but we do not accept them without resevation. In addition to differences reported before, we have observed that sonic vibration or heating to 44°C destroyed the "resistance" antigen for C mice (which do not develop detectable hemagglutinins for DBA cells) but the hemagglutinogen for C57BL survives vibration and heating to 46°C. We then adapted a strain of the DBA tumor so that it would grow in about 50 percent of C57BL mice. A repetition of the above experiments using only the latter

strain of hosts yielded the same general results. We have now obtained a few animals of the isogenic resistant lines C57BL x $H2^d$ and C57BL/ScBs. The DBA tumor grows in the former but not in the latter. The early experiments with these hosts are in line with the previous work. However, there is an elusive quality about the nature of these antigens, and we are not ready to reach conclusions. The matter is important because, if the "resistance" antigen can be distinguished from the hemagglutinogen and localized on the red cell membrane, we have taken a big step toward finding out what it is.

The first stage of the work on the incubation period for tumor resistance following the inoculation of red cells has been completed, and a paper will appear in J.N.C.I. next month. The curve depicting the development of resistance with time is undulating and unlike that described in classical immunology. The unusual feature is that peaks of high resistance alternate with valleys of low resistance after a single inoculum of antigen. The results of inoculating red cells tagged with radioselenium and following the disappearance of selenium from the subcutaneous depot did not support a hypothesis that the first notch in the curve was due to massive breakdown of the red cells during the third week. Three experiments have not supported a hypothesis that the second peak is due to a shifting of "antibody" production from local lymph nodes to the spleen. One experiment has indicated that the massive stimulus associated with tumor inoculation does not result in an undulating curve; instead, the immunity is at a maximum from the 2nd to at least the 13th week. The latter may represent some obscure difference between the normal and tumor cell, or it may be a dosage effect. In either case it may be important, and we are trying to get more information.

Theoretical considerations indicate that, although reciprocal hybrids are generally alike, there are subtle differences between them. This has a general significance in work on immunogenetics, and it had a direct bearing on our previous observation that the mammary tumor agent stimulated a transplanted tumor. Accordingly, Dr. Deringer produced a special type of "doubly reciprocal" F_2 hybrid which furnished a very elegant substrate for detecting the effect of such differences. The completed experiments did not reveal any of the differences that might have been expected, but an unexpected difference was found. There was a correlation in the data between the strain of the tumor and the strain of the paternal grandfather, but this was not ascribable

to the presence of the Y chromosome. Such an effect is unexplained at present, and judgment should be postponed until confirmation is obtained. Is it possible that the sperm transmits an extra-chromosomal factor? A paper reporting this appeared in the October issue of the J.N.C.I.

Several years ago Cloudman reported that mice of strain C3He, obtained by transferring fertilized C3H ova into C57BL mothers, were "tolerant" to a tumor of C57BL origin. If this were true, it would supply an interesting and instructive variation to other cases of "pseudohybridization" or "acquired tolerance." The establishment of strain C3He by Dr. Deringer gave us an opportunity to reinvestigate this. Extensive experiments failed to yield any confirmation of Cloudman's report. A paper reporting this data appeared in the April issue of the J.N.C.I.

I served on the program committee for the annual meeting of the American Association for Cancer Research and participated in the Transplantation Conference of the New York Academy of Sciences as an invited discussor. I retired from the Research Fellowships Board in July after 3 years of service.

A considerable amount of time has been spent in reviewing manuscripts, fellowship applications, and grant-in-aid applications.

Significance: The field with which this work is concerned is so difficult and obscure as to make clear definitions and outlines almost impossible. Nevertheless, there is a slowly growing body of evidence which indicates that there are interactions between tissues and between tissue and its host which involve critical factors in the phenomena of cancer, embryology, and transplantation of normal tissues. A defensible definition of cancer cannot be given today except in descriptive and biologic terms. The terms usually include things related to the experiments that we have been describing. The experiments may be significant in several ways both practical and theoretical.

The most important implication is that some of these results suggest progress toward understanding of what controls the growth of a cancer and how this differs from normal tissues. We have already seen that a tumor's power to overcome host resistance can be altered; its rate of growth, tendency to regress, and tendency to metastasize are all subject to experimental change. The host's reaction can be altered experimentally, not only between similar hosts, but also at

different times and sites in the same host. The changes in the tumor have eluded morphologic, chemical, or serologic definition. One of the antigens which changes the host would be missed by current chemical methods. However, one might attain a biologic description of the factors that sometimes cause spontaneous tumors of man and animals to regress and that control metastasis. It has already been observed in this laboratory (and confirmed elsewhere) that such factors materially effect the outcome of many chemotherapeutic experiments.

Relation to others: There is a complex theoretical interlocking between this work and the work of Drs. Algire, Grobstein, Prehn, and Sanford.

Proposed course: Extension of the main experiments described above. At present, our major need is for tumors with which experiments like the above can be repeated but with certain variations, especially a reversal of the interstrain relationships. We are studying several tumors in a search for suitable ones.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 418
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$32,170	$10,620	$42,790	2	2	4

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2	2	4	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSIST-ANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 418
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Barrett, M.K., Hansen, W. H., and Deringer, M. K.: Failure to observe tolerance to foreign tumor grafts in mice derived from transferred ova. J. Nat. Cancer Inst. 16: 1305-1306, 1956.

(2) _____: The influence of differences between reciprocal hybrids upon tumor transplantation and growth. J. Nat. Cancer Inst. 17: 497-502, 1956.

(3) Barrett, M.K., Hansen, W. H.: Undulations in the time-response curve for tumor immunity after primary immunization with washed erythrocytes. J. Nat. Cancer Inst. In press.

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Appointed to the Editorial Advisory Board of Cancer Research.

(2) Invited to act as chairman and organize the scientific program for the December meeting of the Washington Academy of Surgery.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 419
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. General Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Gastric Cancer Investigations
PROJECT TITLE

7. M. K. Barrett
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: Efforts have been directed at finding methods for producing or studying some of the supposedly etiologic factors in gastric cancer. Many etiologic or predisposing factors have been suggested, but laboratory investigation of them has been held back by a lack of adequate methods for producing them in animals. Our work has comprised a series of attempts to produce starting points from which gastric cancer studies could go forward. In addition, we have attempted to stimulate work in this field generally through the activities of the Gastrointestinal Cancer Committee.

Methods: New techniques of experimental surgery (some developed in this laboratory) and physiology have been used to study peptic

(Use reverse and additional pages, if necessary)

ulceration in rats.

Findings: In keeping with our general ideas regarding tissue specificity, we adopted a hypothesis that the stomach does not digest itself because some "recognition factor" prevents it. A recognition factor or specificity might exist between the enzymes and the tissue, or between some element of the mucus and the tissue, or among all three. If this were so, the mucosa, which is relatively immune to the corrosive action of its own secretions, might be readily attacked by foreign secretion. Under properly controlled conditions gastric juice was transferred between strains of rats. It was found that, although each strain was resistant to its own juice for a period of 4 hours (or more), the glandular mucosa of each was vulnerable to the juice of the other during a period of 3 hours (or less). A manuscript reporting these data was published in Gastroenterology 30: 900-912, 1956.

Significance: The studies on peptic digestion may have far reaching implications, which at present are speculative. One might ask some questions. Is the pepsin specific in an immunogenetic sense? If so, do other enzymes have a biological specificity in the natural state and do they lose this specificity in vitro? (as pepsin may do). Is a different enzyme from pepsin involved in the initial attack upon the mucosa? (If so, the specificity remains). Does the mucus contain a mucopolysaccharide which is antibody-like in function? If so, is this where lysozyme enters the field in peptic ulcer and ulcerative colitis? Does gastric mucosa sometimes contain groups of cells which are immunogenetically aberrant? (Most clones of cells do contain atypical cells). Is the chronic ulcer case an example of this with an area permanently lowered tolerance and the acute ulcer case merely a temporary imbalance of tolerance for transient causes? If so, the first should always be excised for cure; the second may get well without surgery. Is this the reason that nearly all gastric cancers (at the surface) ulcerate? If so, one might be justified in deliberately augmenting ulceration in some small lesions - a long shot. At any rate, this appears to be a new provocative idea in gastric digestion - and, perhaps, in enzymology.

Proposed course: We are planning techniques whereby we may be able to determine whether the specificity of gastric juice for gastric mucosa also extends to unrelated tissues. If the specificity resides in the enzymes, such an extension could be very important. Other attempts will be made if stronger implication of the enzyme can be obtained. So far

our attempts have not been very successful. The action of gastric juice on red cell suspensions has sometimes yielded results which appeared encouraging when judged by visual inspection, but an attempt to substantiate this by following nitrogen partition chemically failed. A large number of observations on the action of gastric juice on intact spleens has yielded specimens which appear harmonious with the working hypothesis, but the morphology is very complicated, and we are trying to enlist the cooperation of a morphologist in evaluating the specimens.

Gastrointestinal Cancer Committee Work: Work on behalf of this committee has been mostly consultative and cooperative during the past year. The two major activities were service on the Program Committee for the 3rd National Cancer Conference and editing the manuscripts for the gastrointestinal panel for that meeting, a service which still is incomplete, and work with the organizing committee for the World Congress of Gastroenterology for 1958, a continuing activity.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 419
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$2,680	$890	$3,570	1/3	0	1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	0	1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 419
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Barrett, M.K.: An apparent "specificity" in digestion of living gastric mucosa in rats. Gastroenterology 30: 900-912, 1956.

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 420 (a)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. General Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the Rous Sarcoma virus. I. Development of the virus-chicken test-system for general use in basic studies on carcinogenesis.
PROJECT TITLE

7. W. Ray Bryan
PRINCIPAL INVESTIGATOR

8. Dorothy Calnan, B. R. Burmester (U.S.D.A., see below)
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Project Description

Objectives:

To improve the biological material (test animals) used in basic studies on Rous virus carcinogenesis and, if possible, to find different genetically homogeneous strains of chickens possessing different degrees of susceptibility to the virus, so that studies can be made of factors influencing host-virus interaction.

Methods employed:

Several genetically inbred lines of chickens have been

(Use reverse and additional pages, if necessary)

developed by workers at the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan, during the past 20 years. In collaborative studies with Dr. B. R. Burmester of that laboratory, a survey was made of 5 of the inbred lines to determine: (a) The relative susceptibilities of the respective lines to the Rous sarcoma virus; (b) the degree of variability (or uniformity) of the responses of individual chickens within lines; and (c) whether the biological results obtained with any one or more of the lines would be superior in any way to those currently obtained with non-inbred chickens from commercial sources.

The actual methods of testing were those which were worked out and reported under this same project heading during previous years.

Major findings: The results of experiments involving a total of over 2000 chickens are still in the process of being analysed and the final quantitative relationships cannot be given at this time. It is clear, however, that extreme differences in susceptibility to the virus do occur among the 5 lines. The line of highest susceptibility and that of lowest susceptibility are separated from each other by about 4 log intervals in the strength of the virus dose required to induce a 50 percent incidence of tumors. Both of these lines show on the whole, greater uniformity among individual chicken responses than is usually obtained with commercial chickens, but there is still some heterogeneity. The nature of the heterogeneity differs, however, from that previously described for commercial chickens. The few chickens that do deviate from the majority of the group in their responses to the virus, differ so markedly that they can be readily determined and separated as belonging to an entirely different population of variates. This may reflect the fact that, although the lines have been inbred brother-to-sister for nearly 20 years, they are not yet considered to be more than about 80 to 88 percent homogeneous (genetically), because of the long interval between generations of chickens and an insufficient number of inbred generations, to date, for complete homogeneity.

The lines of lowest and highest susceptibility constitute extremely valuable test populations for further studies on the virus and on host-virus interaction. The 3 lines of intermediate average susceptibility show considerable heterogeneity comparable with that obtained with commercial stocks. They may represent mixed populations of low and high individuals, rather than true intermediate susceptibility.

Form No. ORP-1
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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 420a
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$10,710	$3,520	$14,230	1/3	1	1-1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1	1 1/3	0

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 420a
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Bryan, W. R.: Biological studies on the Rous Sarcoma virus. IV. Interpretation of tumor-response data involving one inoculation site per chicken. J. Nat. Cancer Inst. 16: 843-863, 1956.

(2) Groupe, V., Rauscher, F. J., Levine, A. S., and Bryan, W. R.: The brain of newly hatched chicks as a host-virus system for biological studies on the Rous sarcoma virus (RSV). J. Nat. Cancer Inst. 16: 865-875, 1956.

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 420b
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. General Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the Rous sarcoma virus. II. Purification and properties of the virus.
PROJECT TITLE

7. John B. Moloney
PRINCIPAL INVESTIGATOR

8. W. Ray Bryan
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To study factors associated with the lability of the virus, with a view toward its better preservation and the recovery of larger amounts of the agent from presently available source tissues.

Methods employed: The increased stability of the virus in citrate buffers (see previous annual reports) and its extreme variability and lability in phosphate buffers represented a point of approach for learning the nature of the factors associated with lability. A systematic study was made of factors which might account for the differences in stability observed between citrate and phosphate buffers. Since lipid oxidation is known to be prevented to some

(Use reverse and additional pages, if necessary)

by citrate, and since products of lipid oxidation are known to inhibit certain other biological phenomena (e.g., cell division; respiration, and glycolysis of tumor cells; certain enzyme reactions), investigations were carried out to determine whether oxidation products derived from the lipids of Rous sarcoma tissue were capable of inactivating the Rous sarcoma virus. The thiobarbituric acid reaction of Kohn and Liversedge was used for the detection and quantitation of oxidized, unstaurated fatty acid products formed by controlled oxidation of phospho-lipids of the tumors and of their microsome fractions. Similar products obtained on oxidation of pure methyl linolenate were used as a control. The residual activity in aliquots of standard Rous sarcoma virus was determined after addition of oxidation products from the above sources, and incubation of the mixtures for 75 minutes at 37°C.

Major findings: It was found that a product (or products) is formed on oxidation of tumor lipids (including those of the microsome fraction, as well as of pure methyl linolenate) and that the concentration of the product is related to the degree of oxidation. Also, it was found that such oxidation products were capable of inactivating the virus in relation to their concentration in phosphate buffers, but that citrate protected the virus up to fairly high concentrations of the inhibitor. Citrate did not protect, however, when the concentrations of oxidation products were excessive, and sufficiently high to saturate the test-system.

A search for antioxidants to be used in addition to citrate is strongly indicated as an important objective toward further purification of the virus, as well as for its production in sufficient quantities for definitive physical and chemical studies.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 420b
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$15,080	$5,300	$21,380	1	1	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 420b
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(3)(Publication of results on the project completed and reported last year under this project heading).

Moloney, J.B.: Biological studies on the Rous sarcoma virus. V. Preparation of improved standard lost of the virus for use in quantitative investigations. J. Nat. Cancer Inst. 16: 877-887, 1956.

(4) Bryan, W. R. and Moloney, J. B.: Rous sarcoma virus - the purification problem. Annals N. Y. Acad. Science (in press).

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 420c
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. General Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the Rous sarcoma virus. III. Further studies on the relation of recoverable virus to tumor initiating dose.
PROJECT TITLE

7. W. Ray Bryan
PRINCIPAL INVESTIGATOR

8. Dorothy Calnan, John B. Moloney
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The quantitative relationship previously demonstrated (see 1955 report) between the dose of virus used to induce a tumor and the amount of virus recoverable on extraction of the tumor suggested certain practical applications as well as explanations of phenomena not previously understood. The objectives of the investigations of the past year were: (a) attempts to increase the virus content of tumor tissue (as an aid to further purification, see project II) by selective serial passage of the virus in chickens; and (b) to determine whether the widely observed phenomena of periodic absence of virus in serial transplants of the tumor tissue could be accounted for by the quantitative relationship stated above, rather than by a change in qualitative state of the

(Use reverse and additional pages, if necessary)

virus (i.e.,"virus masking"), which has been generally assumed to be the explanation of this phenomenon.

Methods employed: (a) A virus passage series was started by inoculation of the strongest available standard virus preparation into 10 chickens and selecting the most rapidly developing tumor as a source of virus for the next serial passage. This same procedure was repeated for all subsequent passages, which now number 27. At each passage, the cell free tumor extract (virus suspension) used for continuing the series was assayed against a standard virus preparation (preserved by freezing and storage in a CO_2 ice chest) in a highly accurate quantitative bioassay experiment.

(b) A tumor produced in a chicken with a minimal effective dose of virus (and which could be expected to contain only very small amounts of virus) was used to start a transplant series, which was further maintained by trocar transfers of small bits of tumor tissue. The original tumor and the tumor selected at each passage for maintenance of the strain was extracted by a standard procedure, freed of cells, and assayed in relation to a standard by quantitative bioassay procedures.

Major findings: (a) The potencies of extracts of the virus-induced tumors increased progressively during the first three serial passages in chickens, to a level of slightly more than 1 log above the potency of the starting virus preparation. This higher level of activity has also been maintained (within limits of random variation due to bioassay error) throughout all further passages of the virus to date. This indicates that the method of selection employed for gathering the source tumor tissue results in virus suspensions 10 to 20 times more potent than are obtained, on an average, without such selection. A very slight upward trend of the potency values with successive passages beyond the 3rd is not statistically significant, but it justifies continuation of the passage series for a longer period of time to determine whether or not the potency may be even further increased.

(b) The original "low-dose" tumor used to start the cell transplant strain contained a very small amount of virus which was on the border line of detection by the methods employed (weaker than the standard by 6 log intervals). The first passage of it showed no activity in the tumor extract (i.e., any virus was below limit of the method), but subsequent passages again showed detectable virus. The tumors were characteristically slower growing, and non-hemorrhagic, as compared with high-dose tumors. The

passages of such characteristic tumors varied considerably in virus content and many failed to show any virus activity at all. Several consecutive passages during a period of 2 months were entirely negative, but subsequent passages again showed activity.

The results essentially duplicate the observations of earlier investigators which have been interpreted as indicating a periodic "masking" of the virus. However, the virus content of the tumor strain was known to be low, on an average, and near the limit of detectability by biological tests in chickens. The periodic "masking" can be readily accounted for in these studies by random fluctuations in individual and in chicken lot sensitivity (known to correspond to a total range of about 3 log intervals, in terms of dosage of virus required to produce a tumor) and the small numbers chickens (10) inoculated for each passage. The assumption of an inactive physical state (i.e., "masked" form) of the virus is therefore unnecessary, and unjustified as an explanation of these results.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 420c
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$16,080	$5,300	$21,380	1	1	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. 420c
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(See publication 3 cited under project II for the virus passage results (a))

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 420d
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. General Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the Rous sarcoma virus. IV. Investigation of factors capable of modifying host response to the virus.
PROJECT TITLE

7. W. Ray Bryan
PRINCIPAL INVESTIGATOR

8. Dr. Vincent Groupe (Rutgers University)
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: For investigating the mode of action of any physiological or pathological factor on biological processes, it is necessary to have access to other factors, or substances, which are capable of modifying this action, either by enhancement or by retardation. The objective of the present investigation was to determine the effect on response to the Rous sarcoma virus of certain substances that were known to modify response to other viruses (e.g., of infectious diseases). The substances tested were hydrocortisone and xerosin. The work was in collaboration with Dr. Vincent Groupe and, for the most part, was carried out in his laboratory at Rutgers University.

(Use reverse and additional pages, if necessary)

Methods: (a) Effects of hydrocortisone. Groups of young chicks were injected daily with various doses of hydrocortisone beginning at various times prior to, and after, inoculation with the Rous sarcoma virus. Alterations in the responses and in the characteristics of tumors were determined in relation to controls injected with solvent, only.

(b) Effects of Xerosin. The procedures were similar to those described for hydrocortisone (above), except that studies were carried out at 3 different levels of virus dosage.

Major findings: (a) Hydrocortisone. When the Rous sarcoma virus was inoculated into chicks pretreated with hydrocortisone, the tumors appeared later than in the controls and were greatly altered in appearance. The altered tumors were sharply circumscribed, firm, whitish, and grew more slowly than the controls which were grossly invasive, soft, hemorrhagic and much larger. When hydrocortisone was discontinued, the altered tumors promptly reverted to typically soft and invasive tumors. When lower doses of hydrocortisone were employed or when injections of hydrocortisone were begun after inoculation with the virus, the tumors which appeared were typical of the controls.

(b) Xerosin: Modifications of the tumor response similar to those described for hydrocortisone were also observed on treatment with Xerosin. The results differed, however, in that Xerosin was still effective when injections of it were begun 24 hours after the virus was inoculated; they differed also in that the tumors did not revert to the control type within a 2 week observation period following the discontinuation of Xerosin treatment.

The most important finding of this investigation was the fact that the degree to which a given concentration of Xerosin was capable of modifying the tumors was a function of the dose of virus which had been used to initiate the tumors. Tumors initiated by the strongest dose of virus were not significantly altered by Xerosin, but those produced by the weakest virus dose were even more modified than in the case of hydrocortisone treatment.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 420d
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$5,360	$1,770	$7,130	1/3	1/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 420d
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(5) Groupe, V., Rauscher, F. J. and Bryan, W. R.: Modification of tumor-response to Rous sarcoma virus (RSV) by hydrocortisone. Proc. Soc. Exper. Biol. Med. 91: 113-117, 1956.

(6) Groupe, V., Rauscher, F. J. and Bryan, W. R.: Suppression and modification of virus-induced Rous sarcoma in chicks by Xerosin. Science 123: 1073-1074, 1956.

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 420e
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. General Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the Rous sarcoma virus. V. Relationship of the virus to other chicken tumor viruses, particularly those of the leukosis complex.
PROJECT TITLE

7. W. Ray Bryan
PRINCIPAL INVESTIGATOR

8. J. W. Beard (Duke University), B. R. Burmester (U.S.D.A.)
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To determine the antigenic similarity. or dissimilarity, of the Rous sarcoma virus in comparison with the following fowl leukosis viruses: (1) myeloblastosis, (2) erythoblastosis, and (3) lymphomatosis.

Methods: This is a 3-way collaboration, led by Dr. J. W. Beard of Duke University, between this laboratory and the laboratories of Dr. Beard and Dr. B. R. Burmester (Regional Poultry Laboratory, U.S.D.A., East Lansing, Michigan). In this laboratory, in which the Rous sarcoma is under investigation, immune sera against the viruses of the various fowl leukoses are being tested (quantitatively) for their ability to neutralize the Rous sarcoma virus.

(Use reverse and additional pages, if necessary)

Also, immune sera against the Rous sarcoma virus are being prepared and supplied to the workers at Duke and East Lansing, for tests against the particular leukosis viruses under investigation in their respective laboratories (i.e., lymphomatosis at East Lansing, and both the myelo- and erythro forms of leukemia, at Duke.)

Major findings: (The investigation has just recently been initiated in this laboratory and the results are inadequate for a report at this time.)

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 420e
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$5,360	$1,770	$7,130	1/3	1/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No items for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. Z20f
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. General Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the Rous sarcoma virus. VI. Investigations of the effects of bacteria on response to the virus and on growth of the tumors.
PROJECT TITLE

7. Dorothy Calnan
PRINCIPAL INVESTIGATOR

8. W. Ray Bryan
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: In spite of meticulous techniques, bacterial contaminations of tumor tissue are at times picked up during the course of serial transplantation for the maintenance of tumor source materials. In some instances marked retardation in the growth of the tumors has been the first signal of such contamination.

A systematic study has been begun of the ability of various bacteria (isolated from such infected tumors and stored in a CO_2 ice chest during the past several years) to specifically alter the response of chickens to the virus, or to modify the course of tumors after they have appeared.

(Use reverse and additional pages, if necessary)

This project has two purposes: First, the search for an organism or product thereof which may have therapeutic value; and second, elucidation of the contribution of bacteria to the variability of biological results obtained in investigations on the Rous sarcoma virus (of importance for technical control of experiments).

Methods employed: The initial procedure being used in this investigation, which has been in progress only for a few months, is that of injecting bacteria directly into tumors induced with the Rous sarcoma virus. The results are determined by comparison with controls in which saline solution, only, is injected into the tumors.

Major findings: The first three organisms investigated were found to produce systemic infections (manifested by generalized peritonitis and pericarditis) and death of the hosts prior to death of the tumor bearing controls. In some instances the tumors were severely damaged, even gangrenous.

The fourth organism, now under investigation, produces an inflammatory reaction which appears to be confined to the injected wing bearing the tumor. The local inflammation subsides after about a week and tumor growth, which is temporarily arrested, is resumed. As a result of this delay, the death of the experimental hosts is later than of the controls, on an average, but some individuals die earlier than the controls, with more widespread metastases than are commonly observed in controls. On the other hand, several (3 out of a group of 20) tumors injected with the bacteria retrogressed completely after growing to a very large size. (Some tumors of controls produced with low doses of virus retrogress spontaneously, but they are slow growing from the start and do not reach a large size before starting to retrogress).

Further investigations involving this interesting and potentially useful bacterium are being continued.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 420f
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$10,710	$3,520	$14,230	1	1/3	1-1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1/3	1 1/3	0

13. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No items for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 421
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. General Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Genetic Studies in Mice A. Tumors in Mice B. Mutations in Mice
PROJECT TITLE

7. Margaret K. Deringer
PRINCIPAL INVESTIGATOR

8. Dr. W. E. Heston Dr. M. K. Barrett
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

A. Tumors in Mice

1. Effect of 4-o-tolylazo-o-toluidine in mice

Objectives:

To study the effect of the subcutaneous inoculation of 4-o-tolylazo-o-toluidine (o-aminoazotoluene) on the incidence of hemangioendotheliomas in strain HR mice, a strain which develops hemangioendotheliomas spontaneously.

(Use reverse and additional pages, if necessary)

Methods employed:

One hundred and twenty-nine strain HR animals (approximately equally distributed as to sex and as to presence or absence of hair) were inoculated subcutaneously in the right axilla with 10 mg. of 4-o-tolylazo-o-toluidine in olive oil. The animals were inoculated at two months of age and each month thereafter until they had received a total of 100 mg. One hundred and fourteen untreated animals were maintained as controls. The animals were observed for the development of tumors and of any unusual lesions.

Major findings:

Strain HR mice are susceptible to the development of spontaneous hemangioendotheliomas and hepatomas. An increase in the incidence of hemangioendotheliomas and of hepatomas has been observed in this strain of mice following the subcutaneous inoculation of 4-o-tolylazo-o-toluidine (o-aminoazotoluene). Hemangioendotheliomas induced with this compound occurred with greater frequency in strain HR mice than has been reported previously in mice of strains A, C3H, C57BL, and BALB/c, with the exception of the BALB/c females. In strain HR, females are more susceptible to the induction of hepatomas by 4-o-tolylazo-o-toluidine than are males.

Significance to the program of the Institute:

This project has added more information concerning the influence of the genetic constitution of the animal on the development of tumors. In this instance the effect of a carcinogen on the incidence of a particular type of tumor, which occurs spontaneously in an inbred strain, has been determined.

Proposed course of project:

This study was completed early in 1956 and the results appeared in the October 1956 issue of the Journal of the National Cancer Institute.

2. Transmission of the mammary tumor agent

Objectives:

To study the effect of the injection of the mammary tumor agent into agent-free strain C3Hf mice during the late stages of pregnancy at which time the agent has been reported to disappear from the blood of mice which carry the agent, and to see if the agent can be transferred through the uterine wall to the fetus (in cooperation with Dr. Heston).

Methods employed:

Groups of strain C3Hf mice have been inoculated intraperitoneally with 0.5 cc. of a 5% cell free extract of a mammary gland tumor from a strain C3H mouse. The groups of animals received inoculations at 1-3, 4-6, and 7-9 days before the birth of the young, immediately following the birth of the young, and when the first litters were weaned. Two groups, maintained as virgins, have received inoculations at 45 and 65 days of age respectively. Untreated female mice have been allowed to produce one litter each and then have been retired at the time at which these litters were weaned. One hundred and ninety-six female offspring of mothers inoculated before birth of the young and fifty-one female offspring of mothers inoculated immediately following the birth of the litter have been kept as virgins. The animals, in this experiment, will be maintained as long as possible and a study of the occurrence of mammary gland tumors and of other types of lesions will be made.

Major findings:

All of the strain C3Hf females in this experiment, except for approximately two thirds of the animals in 2 groups (the group in which the females were inoculated immediately following the birth of the young and the group of females from mothers inoculated following the birth of the litter), have been autopsied. Mammary gland tumors have developed in a small number of the females of all groups but in no group was the incidence as high as that observed in the strain C3Hf females of the breeding colony maintained in this laboratory. It is apparent also that the average age at which the mammary tumors appeared was not decreased by the presence of

the mammary tumor agent. It is evident, from the data obtained in this experiment, that the agent was not transferred through the uterine wall of the inoculated mothers to their offspring.

Significance to the program of the Institute:

This experiment will contribute further knowledge concerning the mode of action of one of the factors, the mammary tumor milk agent, which is responsible for the development of mammary gland tumors in certain strains of mice.

Proposed course of project:

It is anticipated that this project will be completed in 1957. The results obtained will then be prepared for publication.

3. Mammary gland tumors in C3He mice

Objectives:

To study the incidence of mammary gland tumors in virgin females, in breeding females, and in forced-bred females, and in stilbestrol treated males of strain C3He (a substrain developed in this laboratory by the transfer of fertilized ova from a strain C3H mouse to a strain C57BL mouse).

Methods employed:

One hundred and five strain C3He females have been set aside as virgins. Ninety-nine C3He females have been forced-bred. One hundred and five males have been inoculated subcutaneously at the right axilla with 6-8 mg. pellets of 10% stilbestrol in cholesterol. The breeding females, from which data will be obtained, are those which are used to maintain the colony of strain C3He mice in this laboratory.

Major findings:

Only a few of the animals in this experiment have been autopsied. These animals either had developed a tumor or were moribund. None of the virgin strain C3He females autopsied have developed a mammary gland tumor. Mammary gland tumors have been observed in about 36% of the forced-bred females and in about 64% of the males treated with stilbestrol that have been autopsied. At autopsy a number of the tumors, present in the treated males, were observed to contain a milky fluid. A marked lactation effect was observed in the sections of the tumors. Three of the tumors are being transplanted in strain C3He males and females in order to determine whether this effect will persist in successive transplant generations of the tumors.

Significance to the program of the Institute:

This experiment will supply further information concerning the development of mammary gland tumors in a strain of mice deprived of the mammary tumor milk agent. This was accomplished by the transfer of fertilized ova from an inbred strain of mice carrying the agent to another strain without the agent.

Proposed course of project:

This experiment will probably be completed during 1957.

4. Determination of presence or absence of the mammary tumor agent by foster-nursing

Objectives:

To determine whether a mammary tumor agent is present in strain BL (Bagg L) and in strain SWR (Swiss) mice (in cooperation with Dr. Heston).

Methods employed:

One hundred newborn female mice of strains C3Hf or C3He have been foster-nursed on strain BL and one hundred on strain SWR females. The foster-nursed animals are being maintained as virgins and are being observed for the development of mammary gland tumors.

Major findings:

One-fourth of the strain C3Hf and strain C3He females, which have foster-nursed the strain BL females have been autopsied. An incidence of mammary gland tumors of 42.3% appearing at an average age of 13.7 months has been observed. Nearly one-third of the females which have foster-nursed the strain SWR females have been autopsied. One female has developed a mammary gland tumor at 25 months of age. These preliminary data seem to indicate the presence of a mammary tumor agent in the strain BL but not in the strain SWR mice.

Significance to the program of the Institute:

This experiment will provide more information concerning the characteristics of the two inbred strains. This will increase their usefulness for experimental purposes.

Proposed course of project:

It is anticipated that this project will be completed in 1957.

5. Production of a strain of mice free of the mammary tumor agent

Objectives:

To produce a second mammary tumor agent-free strain of mice from animals developed from transferred ova.

Methods employed:

The fertilized ova of strain DBA/2 mice are transferred to strain C57BL mice which have been bred.

Major findings:

No results at the present time.

Significance to the program of the Institute:

The production of another mammary tumor agent-free strain of mice will provide a strain which will be useful in various types of experiments in which studies of mammary gland tumors are made.

Proposed course of project:

Fertilized ova will be transferred from strain DBA/2 females to strain C57BL females until a pair of animals, from which an inbred line can be started, is obtained. When the line is established and sufficient animals are available, various experiments, utilizing them, will be performed.

6. Immunogenetic experiments

Objectives:

To produce special types of animals for collaborative experiments with Dr. M. K. Barrett (see Dr. Barrett's report for details).

B. Mutations in Mice

1. Mutant affecting locomotion

Objectives:

To test a mutant, phenotypically like waltzer, which arose in strain A mice (in cooperation with Dr. Heston).

Methods employed:

Strain A males, showing the "waltzing" character, have been outcrossed to strain 1194 females. F_1, F_2, reciprocal backcross generations, and second backcross generations have been produced. Data are collected regarding the presence of the character in these different groups of animals in the hope of establishing the mode of inheritance of this character.

Major findings:

The data indicate that this character, designated waltzer for the present, is inherited as a simple recessive. The gene exhibits some lethal effect, as indicated in the failure to obtain the expected 1:1 ratio in the segregation of the backcross offspring.

Significance to the program of the Institute:

The study of this mutant may be of significance in providing another known character which will be of use in the linkage studies of factors influencing susceptibility to various types of tumors.

Proposed course of project:

Crosses between strain A mice, exhibiting this "waltzing" character and other strains exhibiting similar characters (as for example, shaker-2), are planned for the future.

2. Mutant affecting character of the coat

Objectives:

To test a mutant, phenotypically like caracul, which arose in strain C3Hf mice in this laboratory.

Methods employed:

At the present time, it is assumed that this coat mutation in strain C3Hf mice is caracul, which behaves as a dominant character. Matings have been made using animals which show the character attempting to produce animals homozygous for the character. When this has been accomplished, crosses will be made between these animals and animals of other strains exhibiting known characters affecting the coat of the animals (as for example, waved-2), in order to establish the identity of the gene in question.

Major findings:

No results have been obtained.

Significance of the program to the Institute:

The study of this mutant may be of significance in providing another character which will be of use in the linkage studies of factors influencing susceptibility to various types of tumors.

Proposed course of project:

The mutant strain C3Hf mice will be outcrossed to a strain exhibiting another character affecting the coat of the animals, in order to determine whether or not this character (designated caracul for the present) is identical with a known character. F_1, F_2, and reciprocal backcross generations will be produced. Data on the identity of and the mode of inheritance of the character will be obtained. This project will continue through 1957.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 421
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$16,080	$5,300	$21,380	1	1	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 421
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Deringer, M.K. and Heston, W.E.: Abnormalities of urogenital system in strain A x C line 9935 rats. Proc. Soc. Exp. Biol. and Med. 91: 312-314, 1956

(2) Barrett, M. K., Hansen, W. H., and Deringer, M. K.: Failure to observe tolerance of foreign tumor implants in mice derived from transferred ova. J. Nat. Cancer Inst. 16: 1305-1306, 1956

(3) Heston, W. H., Deringer, M. K., and Dunn, T. B.: Further studies on the relationship between the genotype and the mammary tumor agent in mice. J. Nat. Cancer Inst. 16: 1309-1334, 1956

(4) Barrett, M. K., Hansen, W. H., and Deringer, M. K.: The influence of differences between reciprocal hybrids upon tumor transplantation and growth. J. Nat. Cancer Inst. 17: 497-502, 1956

(continued)

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Project 421 - Part C, Publications (cont.)

(5) Deringer, M. K.: Effect of the injection of 4-o-tolylazo-o-toluidine (o-aminoazotoluene) subcutaneously in strain HR mice. J. Nat. Cancer Inst. 17: 533-539, 1956

(6) Dunn, T. B., Heston, W. E., and Deringer, M. K.: Subcutaneous sarcomas in strains C3H and C57BL female mice, and F_1 and backcross hybrids of these strains. J. Nat. Cancer Inst. 17: 639-655, 1956

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 423
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. General Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Developmental Physiology
PROJECT TITLE

7. Clifford Grobstein
PRINCIPAL INVESTIGATOR

8. Dr. Robert Auerbach (Research Fellow)
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

To increase understanding of the interaction of tissues and the components of rudiments in normal development as these relate to the differentiation of new cell types, and to apply information obtained to pathological problems, in particular to problems of tumorigenesis and malignancy.

Methods:

Developing rudiments of mouse organs, e.g., kidney, salivary gland, and axial skeleton, are separated into their components and

(Use reverse and additional pages, if necessary)

cultured in isolation and in various recombinations to determine the degree to which their development is interdependent and the nature of the interactive mechanisms involved. Development is observed in vitro and/or on reimplantation into the anterior chamber of the eye.

Major findings:

Continued study of trans-filter induction between embryonic spinal cord and metanephrogenic mesenchyme has lead to the conclusions that the agent involved is not cytoplasmic on the one hand, nor transmissible through cellophane on the other. The first conclusion arises from the electron microscopic finding, in collaboration with Dr. A. J. Dalton, that cytoplasmic penetration is completely excluded at a filter porosity which still permits inductive activity to cross- though in reduced intensity. The second conclusion stems from experiments with three filter layers in which the middle layer is replaced by cellophane containing a small hole. Tubule induction occurs only in close association with the hole, indicating that the activity is blocked by cellophane but can pass across a "free space" of 20-30 microns between the two filter layers. Other experiments with multi-layered filters indicate that maximum transmission of the activity lies in the vicinity of 80 microns.

These results are in harmony with the earlier hypothesis that inductive activity resides in large molecular materials which are produced by the cells and ordinarily confined to their immediate vicinity. Efforts are in progress to collect such materials, free of cells, by continued exposure of relatively large quantities of disaggregated embryonic dorsal spinal cord to small volumes of various defined and semi-defined media. In connection with these studies, in collaboration with Dr. R. Auerbach, apparatus and procedures have been worked out for disaggregation of embryonic tissues, and their maintenance for extended periods in the disaggregated state under physiological conditions. In developing and standardizing these procedures the finding has been made that both dorsal spinal cord and metanephrogenic mesenchyme can still participate in the inductive process upon reaggregation after short-term disaggregation to the cell level.

In related studies, employing a new procedure for organ culture in the absence of a clot, it has been found that neither the fully defined medium of Eagle or Earle can support morphogenesis or survival of mouse metanephric rudiments,

and that various large molecular supplements to these media give very different effects. Horse or human serum supplementation promotes morphogenesis, chick embryo juice supplementation promotes survival and rapid cellular migration, but yields limited morphogenesis. Serum albumens give serum-like effects while serum globulins and dextrans seem inactive. No supplementation has so far duplicated the favorable effects of the undefined medium routinely employed.

Continuing study of the formation of epithelial tubules in vivo by metanephrogenic mesenchyme, in collaboration with Mr. George Parker, has shown tubule formation to occur in limited degree in the subcutaneous tissue of the mouse, and vigorously in the body wall of the embryonic chick. On the chick chorioallantoic membrane, however, tubule formation does not occur even when the graft mass is large enough to insure regular survival and incorporation. In the eye, where tubule formation in the absence of known inductors was first seen, further study shows that graft and iris mutually infiltrate each other and tubules arise during this intimate association. When grafts are made into partially iridectomized eyes, those which lodge on the lens capsule form tubules, but those on the cornea do not. The results suggest that iris and lens capsule have tubule-inducing effects in the general eye environment, though they are inactive when tested in vitro. On the other hand, the general eye environment is not itself inductive when grafts are on the cornea. Thus, it appears that at least two factors are involved in tubule formation in the eye.

Significance to cancer research:

The conception which has arisen from results to date, and which is now taken as a working hypothesis for further work, attributes importance to large molecular intercellular materials as determinants of cell motility, cell shape, differentiation and other aspects of tissue behavior and architecture. Since tumorigenesis involves alteration of tissue behavior and architecture, a natural corollary to the working hypothesis is the assumption that large molecular intercellular materials undergo change in tumorigenesis. The fruitfulness of this suggestion, which has been made previously on entirely different grounds, can only be judged in the light of future studies.

Proposed course of project:

Continued attempts are planned to obtain tubule-inducing activity in cell-free solutions. It is not, however, intended to pursue this beyond a few obvious and relatively simple procedures. If these prove negative the attempts will be dropped pending new leads from other sources.

When the present line of work has been carried as far as now appears feasible, attention probably will be shifted back to the focus of earlier studies - the nature of the changes involved in the origin and stabilization of new properties in developing tissues. The concept will be tested that changes in intercellular materials are as important as cellular changes in these processes, both normal and malignant. The procedures will involve disaggregation of tissues undergoing property changes, and study of the effects of simple disaggregation or of reaggregation in various macromolecular environments and in association with various other cell types, both normal and malignant.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 423
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$40,180	$13,270	$53,450	1	4	5

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	4	5	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications. 15. 423
SERIAL NUMBER

16. LIST OF PUBLICATIONS RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

1. Grobstein, C.: Inductive interaction in the development of the mouse metanephros. J. Exp. Zool. 130: 319-340. 1955.

2. Grobstein, C.: Transfilter induction of tubules in mouse metanephrogenic mesenchyme. Exper. Cell Res. 10: 424-440. 1956.

3. Grobstein, C.: Inductive tissue interaction in development. In press. Advances in Cancer Research, Vol. IV.

4. Grobstein, C.: Kidney tubule induction in mouse metanephrogenic mesenchyme without cytoplasmic contact. (With A. J. Dalton). J. Exp. Zool. In press.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 427
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia Studies Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the etiology and chemotherapy of experimental lymphomas
PROJECT TITLE

7. Lloyd W. Law
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

1) Studies of the etiology and pathogenesis of experimental lymphomas.

A. The role of the thymus in spontaneous cases.

Grafts of AKR thymic fragments (30 day) have been shown to bring about an earlier appearance of lymphomas in (AKRxC3H)F_1 mice. C3H thymuses exhibited no such influence. Additional studies are now in progress to study a) influence of thymectomy in these F_1 hybrids (preliminary data showed an influence only on age at death from leukemia in thymectomized mice), b) influence of thymic grafts in thymectomized F_1 mice, c) influence of 1 to 10 day AKR x C3H thymuses in contrast to 30 day thymic fragments. (In collaboration with Dr. M. Potter)

(Use reverse and additional pages, if necessary)

B. Influence of grafted thymic tissue following exposure of recipient mice to x-radiation.

Additional data are now available concerning the influence of C57BL and A strain thymic tissue grafted into thymectomized, irradiated (4 x 225 r, q7) C57BL x A F_1 mice:

1. Thymectomy at one month of age completely inhibits the production of lymphocytic neoplasms (90% in controls). Type A and B reticular neoplasms appear not to be so influenced.

2. More than 30% of these F_1 mice with C57BL (1 to 10 day) thymuses develop lymphocytic neoplasms in the graft. A smaller number of neoplasms develop from strain A thymuses.

It is clear that irradiation "conditions" these F_1 test mice so that they have the potentialities of transforming normal cells to leukemic cells only if thymic tissue (C57BL or A) is present. The neoplasms arise within the grafted tissue. Transplantation studies of the induced neoplasms in A, C57BL and (C57BL x A)F_1 mice reveal that in some cases the neoplasms are C57BL genetically and in other instances F_1 genetically, corroborating the work of Kaplan that round cells not previously exposed to the X-rays do become leukemic in a "conditioned" host, and extending these observations to show that F_1 round cells may repopulate the graft and under some "sphere of influence", probably epithelial tissue of the thymus and become leukemic. In this connection it is hoped to investigate fully the role of a lymphocytosis stimulating substance (LSS) formed by Metcalf to be present in epithelial tissue of thymuses of certain high-leukemic lines (AKR).

3. The occurrence of leukemic chimeras, studied by Uphoff, provides information that bone marrow plays a part in the irradiation-induced disease. Bone marrow from high-leukemic lines, particularly AKR, was used to protect (homologous) certain unrelated F_1 hybrid mice given 700-900 r. It was found in certain of the few survivors that the leukemia which developed (e.g. in BAF_1 mice with AKR bone marrow) were genetically of the bone-marrow donor type (H-2K). This is further proof of the reseeding of the bone marrow of irradiated hosts and provides a model to gain information of the role of bone marrow in thymic leukemias.

4. In an attempt to gain information on the persistence of the "post-irradiation state" in BAF_1 mice, thymic grafts have been introduced into these mice at 14, 21 and 28 days following irradiation. 8 Mice to date (3 introduced at 28 days) have given rise to lymphocytic neoplasms (in the graft). (Collaborative work with Dr. M. Potter)

C. Characterization of a high-leukemic C3H subline.

In 1953 we obtained from Dr. H. J. Figge a subline of mice, C3H/Fg, which were observed to develop a high incidence of lymphomas. We confirmed these results of Figge and found also that parotid gland neoplasms also made their appearance. Due to the complications of mammary tumors in this line, we foster-nursed a litter of F95 obtained by Caesarian section and established the subline C3Hf/Fg. Offspring from a single pair mating ♀ 18706 x ♂ 18707 have been obtained through 5 further inbred generations with the following interesting data obtained:

Sex	No. mice	Lymphomas No.	Lymphomas %	Mean Age at death Mos.
♀♀	73	59	80.9	
♂♂	45	18	40.0	13.2(5-22)

51% of the neoplasms of blood forming organs are lymphocytic neoplasms, 6% type A neoplasm, 17% type B, 11% granulocytic and 13% plasma cell neoplasms.

F_1 mice obtained through crossing with C3Hf/Lw and STOLI show an intermediate incidence and reciprocal F_1s are alike in incidence and mean age at death from the neoplasms.

Attempts are being made to assess the role of a cell-free agent (Gross) and genetic susceptibility in the occurrence of a high-leukemic C3H subline:

a) Cell free extracts from numerous (10 to date) spontaneous and transplantable lymphomas have been inoculated into C3H/Bittner(Z) mice. No induced neoplasms have been encountered to date.

b) F_1 mice, (C3Hf/Fg x C3Hf/Lw) and 1st backcross mice (F_1 x C3Hf/Lw) have been obtained in order to run a "progeny test" of 50 1st backcross males mated with the low-leukemic C3Hf/Lw females. The incidence of lymphomas in 50 families of 2nd backcross offspring will be obtained in order to determine the genetic potentialities of the 1st backcross mice, and thus the role of genetic factors in the establishment of this high-leukemic, C3Hf/Fg, subline.

Thymectomy has been found not to influence the incidence of lymphomas, but probably, the mean age at death in this subline.

X-radiation (90 r x 4, q7 days) increases the number of thymic lymphocytic neoplasms.

D. The role of cell-free filtrates in the induction of lymphomas and other neoplasms.

a) Most of the test mice used in a study reported last year, C3Hf/Lw, (C3H x AKR)F_1 and (C3H x C3H/Fg)F_1 given cell-free extracts particularly obtained from AKR leukemic tissues, have now been autopsied. These subsequent results do not change the picture presented in that report. It is of interest to note a lower incidence of lymphomas in the (C3H x AKR)F_1 mice receiving filtrates than in the litter-mate controls. Many unusual neoplasms make their appearance in this specific F_1 cross.

b) In an attempt to assess the role subline differences may play in response to hypothetical leukemogenic, cell-free agents, there were obtained both from Dr. Bittner and Dr. Gross nuclei of their inbred sublines. We have continued to breed these brother x sister in order, 1) to observe them for tumor incidence and 2) to use them on recipient mice along with the subline used previously C3Hf/Lw for cell-free filtrates from leukemic AKR mice. The following interesting information, though preliminary, is now available:

1. A goodly number of lymphomas have been seen among the 5 families of C3H/Bittner (Z) mice and also among 1 family of C3H/Gross mice.

In family III, for example, 2 of 5 mice obtained from Bittner had lymphomas at 14 and 18 months. Among 20 litters obtained from this F78 generation constituting 64 mice, there have been 6 lymphomas (11 to 19 months) and curiously 5 pleomorphic parotid gland neoplasms. Since many of these mice are still alive, it is impossible to state the incidence of lymphomas, but it is clear that it is much higher than reported heretofore.

2. Extracts and cell-free filtrates from 14 spontaneous AKR leukemias have been inoculated into 387 Z mice and the same material into 102 C3Hf/Lw, less than 12 hours of age with the following results:

Strain	No. mice	Lymphomas No.	Lymphomas %	Parotid Gld. Neoplasms No.	Parotid Gld. Neoplasms %
a) Exptl. Z	387(159)	10	6.9	5	3.2
b) Controls	63	3	4.8		None
C3Hf/Lw	102(86)	None			

Lymphomas in a) appeared at 3,4,4,5,5,7,9,9,9 and 10 months
in b) at 11, 13 and 14 months.

It is of interest to note the 60% mortality in Z mice receiving filtrates and less than 20% in C3Hf/Lw receiving identical material.

Several lymphomas in the experimental series were obtained early using 9500 RPM centrifugates. These resulted from transfer of viable cells and grew progressively, following transplant only in AKR mice.

Suitable breeding colonies of AKR and Z strain mice are in the process of being established in order to transfer fertilized ova from Z strain to AKR mothers.

E. Studies of certain inhibitory (repressive) factors in induced and spontaneous lymphomas.

1. The influence of intact bone-marrow cells.

a) Data are now complete in the study of the influence of AKR and C3H bone marrow in spontaneous and radiation-induced lymphomas of (C3H x AKR)F_1 mice. This was a collaborative study with Dr. Egon Lorenz and Dr. Charles Congdon (now at Oak Ridge) and will be published shortly. Last year's annual report gave the pertinent findings.

b) Two groups of mice DBA/2 and BAF_1 have been thymectomized and irradiated (fractionated). Both groups give a relatively high incidence of type A and B reticular neoplasms (Dunn's classification), if lymphocytic neoplasms are prevented by thymectomy. Intact bone marrow cells (isologous transfer), one long bone equivalent, are given following the last of 4 radiation treatments. It is too early to determine, in this series, 1) the influence of fractionated radiation as the induction of these neoplasms and 2) the possibility of repressive action of bone marrow.

2. Studies on maternal resistance factor.

Mac Dowell first described the presence in a low-leukemic strain, STOLI, of a factor transmitted in utero and through the mother's milk which prevented the occurrence of lymphomas in high-leukemic C58 and in hybrids involving this strain. Old STOLI females, but not young (up to 6 months of age) transmitted this influence. We had found also that this influence was found in the low-leukemic NH strains, but not others: C3H, C57BL etc.

Data are now available to indicate that the expression of this influence is dependent upon the genotype of the test animal. The incidence of lymphomas is decreased and the age at death from the disease is lengthened in C58 or (STOLI x C58)F_1 mice reared by old STOLI mothers, whereas there is little or no influence on the incidence or mean age at death from lymphocytic neoplasms using the high-leukemic AKR strain or an F_1 hybrid (STOLI x AKR). Litter mates were divided among young and old STOLI mothers and AKRs and C58s fostered on the same females. Pertinent data for the inbred strains are given:

Strain	Total No.	Incidence of lymphomas		Age at Death	
		Old females	Young females	Old females	Young females
C58	45	41.7%	90.5%	15.1 mo.	10.7 mo.
AKR	113	77.7%	98.3%	11.8 mo.	10.1 mo.

2) Studies of mechanisms involved in inhibition of leukemic cell growth.

A. Collaborative studies are continuing with Dr. Arnold Welch, Yale University, with certain pyrimidine antagonists, particularly 6-Azauracil.

Intensive efforts to produce a 6-Azauracil-resistant subline of the lymphocytic neoplasm L4946 have failed. There is some evidence however that resistance has developed in the plasma cell neoplasm, 70429, to this compound. It is hoped soon to have available the riboside of this compound since as shown by Welch and Colleagues this is the more effective compound in bacterial inhibition and in tissue cultures of neoplastic cells (S-180) where 6-Azauracil is ineffective. The riboside has been shown to enter both sensitive and resistant bacterial cells, but in the resistant cells is found only in the acid soluble fraction - not in the nucleic acids. Thus our main objectives at the moment are of finding other neoplasms more sensitive to this compound, and the subsequent development of resistance.

B. Work is continuing in collaboration with Dr. Charles Nichol, now at Roswell Park Memorial Institute.

1) An *in vitro* system using ascitic forms of certain lymphomas: L-1210, L-4946 and 70429 has been developed and standardized and interesting differences between sensitive cells and those resistant to antifolics, antipurines and antiglutamines have been found as regards a) their CF content, b) capacity to alter PGA to compounds measurable as CF and c) differences in sensitivity to PGA antagonists.

This system appears to lend itself well to cell-free studies and the role of permeability, active transport and differences in specific enzyme systems as they relate to resistance.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 427
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$44,180	$14,530	$58,710	2½	3	5½

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2 1/2	3	5 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 427
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Law, L. W.: Differences between cancers in terms of evolution of drug resistance. Cancer Research 16(7): 698-716, 1956.

Law, L. W., and M. Potter: The behavior in transplant of lymphocytic neoplasms arising from parental thymic grafts in irradiated, thymectomized hybrid mice. Proc. Nat. Acad. Sciences 42(3): 160-167, 1956.

Shacter, B., and L. W. Law: Influence of amethopterin on tumor growth and liver glutathione levels of mice bearing lymphocytic leukemias. J. Nat. Cancer Inst. 17(3): 391-398, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Recipient 1955 AAAS- Anne Frankel Rosenthal Award in Cancer Research.

Elected to Board of Directors, American Association for Cancer Research

Chosen member Advisory Committee on the Therapy of Cancer, American Cancer Society.

Chosen member Advisory Board, Childrens Cancer Fund of New York.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 427(a)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia Studies Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the etiology and chemotherapy of experimental lymphomas
PROJECT TITLE

7. Michael Potter
PRINCIPAL INVESTIGATOR

8. Dr. Thelma B. Dunn, Dr. Clyde Dawe, Dr. John L. Fahey
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

I Leukemogenesis:

A. Thymic Graft Experiments

Experiments on the role of the AKR thymic graft in thymectomized and non-thymectomized $(C3HxAKR)F_1$ hybrid mice is in progress; this work is being done with Dr. L. W. Law. A second large series of mice $(C57BLxA)F_1$ hybrid mice, which have been thymectomized, irradiated and grafted with 1-6 day old C57BL thymic grafts is in progress, this work also is described in the report of Dr. L. W. Law. This work involves the detection of thymic graft neoplasms, and the study of their transplant behavior, through several transfer generations.

(Use reverse and additional pages, if necessary)

B. Rf Strain Mice

This strain, reported by Furth to have a moderate incidence of granulocytic leukemia has been established in this laboratory. Attempts to establish neoplasms from this strain in transplant have failed and it has been reported to us that this strain has in all probability been contaminated by outcrossing. At the present time Rf mice are being inbred, and 4th generation mice are currently breeding. A high incidence of spontaneous leukemia, over 40% has been observed, and numerous granulocytic leukemias have been seen. It is hoped that there will be genetic uniformity by the 10th brother-sister generation.

C. Effect of Serial Thymectomy on the Incidence of Leukemia in AKR Mice.

AKR mice have been thymectomized at one month of age, and the progeny of these animals have been thymectomized. This has been carried out for 6 generations. The progeny of the 6th serial thymectomized generation has been set out, and are being observed for leukemic incidence. This experiment has been done to establish the role of a possible transmissable agent. Thymectomy reduces leukemia incidence drastically. Thus by removing the tissue in which an agent presumably would multiply, it may be possible to reduce its quantity over a period of time; and thus lower leukemic incidence.

D. Crude tobacco smoke condensate

DBAf/2 mice painted for 3-4 months with crude tobacco smoke condensate failed to develope leukemia in the first 12 months of observation.

E. Comparative action of x-radiation and methylcholanthrene on the induction of various lymphomata in DBAf/2 mice.

This study as mentioned in last years report is still in progress. One of the main technical difficulties is in establishing the optimally effective dose of irradiation. Kaplan has demonstrated that the interval between the fractionated doses of X-ray can be extended as long as 16 days with C57Black mice and the leukemic incidence is equivalent with that found in groups irradiated every 4 or 8 days. This observation has been confirmed, and will be used as the treatment schedule with DBAf/2 mice. The role of gonadectomy and thymectomy is being studied.

Dr. Thelma Dunn is autopsying the mice in this experiment. A review of the pathologic findings will be undertaken this year.

F. Estrogen induced lymphocytic neoplasms in C3H sublines
Preliminary experiments with C3H/Bi, (Z) and C3Hf/Lw mice which were treated with estradiol benzoate over a 4 month period failed to develope thymic leukemia. The use of sesame oil provided a considerable technical difficulty in performing this work. This experiment will be repeated employing a pellet of estradiol dipropionate.

G. (DBAxCE)F_1 and (CE x DBA)F_1 hybrid mice failed to develop a high incidence of leukemia following methylcholanthrene painting. These studies are being discontinued.

II A. The Utilization of a Spectrum of Reticular Neoplasms of the Mouse.
The spectrum including all of the major types of reticular neoplasms of the Dunn Classification has been established in transplant. (see table) This spectrum has been developed in two common strains of mice DBAf/2 and C3H/HE, thus making it readily available to most workers in the field of chemotherapy. The recent isolation of a granulocytic leukemia in the DBAf/2 mouse is an example of the practicality of this spectrum. This group of neoplasms has proved to be a valuable system for the study of response to antimetabolites and work is in progress on further characterization.

Certain notable findings have already been described and will appear in publications now in press. These are: The occurrence of myeloma globulins in the serum of mice bearing the two plasma cell neoplasms X5563, and X5649, and the histamine production of the mast cell neoplasm 815. In cooperation with Dr. Clyde Dawe, the lymphocytic neoplasm 388 has been established by him in tissue culture. It is expected from present findings that this will lend itself to new techniques for the study of drug resistance.

Interesting results have been found, essentially: 1) a differential response to the antipurines, 8 azaguanine, 6 mercaptopurine, and thioguanine, 2) a different quantitative response to Amethopterin, 3) different modes of developing resistance to various antimetabolites, 4) unusual responses to combinations of antimetabolites, e.g. DON and N methyl formamide, DON and 8 azaguanine, DON and 6 mercaptopurine.

A second myeloma globulin producing plasma cell neoplasm X5649 has been established in transplant. This globulin is quite different from that produced by plasma cell neoplasm X5563. A continued search is being made for new plasma cell neoplasms in C3H mice. Characterization of the abnormal proteins is being carried out with Dr. John L. Fahey. Cooperation with Dr. Fahey for his studies of protein synthesis with these tumors is being accomplished. Further studies on the nature of serum protein changes in other lymphomata of the mouse are being studied.

Neoplasm	Type	Strain	Transfer Generation	Ascites	Ameth.	Antipurine	DON
P288 //	LN	DBAf/2	78	+	+++	+	-
P335	LN	"	75	+	+	+	+
P388	LN	"	78	+	++++	+	-
P228 ʃ	A	"	34	+	+	-	-
P329 ʃ	A	"	26	+	+++	+++	+++
P541 ʃ	A	"	14	+	?	?	?
P1081 †	G	"	2	No	?	?	?
P815 ‡	M	"	18	+	-	-	+++
P195	B	"	18	No	?	?	?
70429	PC	C3Hf/HE	44	+	++	-	++++
X5563*	PC	"	6	No	?	?	?
X5649*	PC	"	2	No	?	?	?

LN=Lymphocytic neoplasm. A=type A reticulum cell sarcoma. G=granulocytic leukemia. M=mast cell neoplasm. B=type B reticulum cell sarcoma. PC=plasma cell neoplasm.

* Produces myeloma globulin
† Chloroma
‡ Produces large quantities of histamine
ʃ Produces hematoidin crystals
// Tetraploid

B. Studies on the mechanism of the development of resistance to Azaserine, DON, Amethopterin in neoplasms 70429, 815, and 288.

These three neoplasms have proved to be ideal laboratory tools for studies of the mechanism of the development of resistance to antimetabolites. Certain basic patterns of resistance have been found.

1) The Azaserine and DON resistant 70429 populations, emerge in a single transfer generation history, either during continuous therapy, or in "recurrent" populations which appear several months after the cessation of therapy. They are not resistant to Amethopterin.

2) Amethopterin resistant 70429 populations emerge after 20-30 days of continuous exposure to amethopterin. They are not cross resistant to the glutamine antagonists.

3) DON resistant 815 populations arise after two and three generations of continuous exposure.

4) An amethopterin resistant population has been obtained from the tetraploid lymphocytic neoplasm P288. This population was derived after several transfer generations. This neoplasm has been no more reluctant than other diploid lymphocytic neoplasms to develop amethopterin resistance. Further studies on the mechanism of resistance to amethopterin are in progress.

Comparisons of the rate of development of resistance in diploid and tetraploid tumorsis of particular interest, since some understanding may be obtained from these interesting genetic tools.

The ease of obtaining Azaserine resistant 70429 populations is related to two possible mechanisms.

1) The stem line population is mixed and already contains a number of resistant cells.

2) The change from S to R is a simple biologic mechanism i.e. a simple gene mutation, either at the enzyme or cell surface level.

Further studies are needed to clarify these points. Such studies are meticulous, and will require the isolation of clones from single cells, which are highly sensitive.

If resistance developes with equal frequency in the single cell line of 70429-sensitive, then there will be irrefutable evidence that the change from S to R, which has been shown already to be genetically stable and heritable, is a somatic mutation. It will then remain to determine the site of the mutation. (cell permeability, gene-enzyme alteration or adaptive enzyme production). Work is in progress with Dr. E. Anderson of this laboratory on the biochemical nature of azaserine resistance.

The study of the recurrent neoplasms may have already provided a simple means of solution since two of 6 recurrences thus far studied are highly sensitive to azaserine.

Studies are already in progress to quantitate the frequency of development of resistance by employing means which will prevent resistance such as the addition of a "sequential block", e.g. Amethopterin. The standardization of resistance frequency, and the underlying biologic principles involved in the resistance mechanism are some of the most important principles in understanding how to control malignant populations. It is felt this area needs great emphasis. Many seemingly unrelated biologic phenomena may well be involved. Examples of this are, 1) the role of cortisone. (Cortisone has been shown to inhibit neoplasm 70429 by slowing cellular accumulation and infiltration), 2) hyaluronidase.

There is a great need for further antagonists in the de novo synthetic pathway of the purine ring as described in the work of Buchanan. One particular substance is an aspartic acid analogue.

Parke Davis and Company have supplied us with interesting possible analogues and the possible inhibitory effect of these substances are being studied in neoplasm 70429. The addition of a third substance will allow a more complete description of resistance, since the two independently acting classes of antagonists, the glutamine and folic acid analogues in this tumor are not cross-resistant.

C. Cooperation with Dr. Clyde Dawe, on the Effect of growth in Tissue Culture of the Lymphocytic Neoplasm P388.

The lymphocytic population in vitro has transformed by an unknown mechanism to a neoplasm resembling a type A reticulum cell sarcoma. Preliminary studies of the chromosome modality of the two neoplasms has revealed the stem line lymphocytic neoplasm is diploid, whereas the transformed line is a near tetraploid. The

transformed tumor behaves as a DBA/2 neoplasm. This is important since the P388 prior to the introduction in vitro had been maintained in (DBAxC)F_1 hybrid mice. Further studies on the biologic nature of this transformation are being studied by employing chromosome counts, drug sensitivity, growth curves and transplant behavior in compatible and foreign strains.

In summary, experiments are in progress to, 1) establish the biologic patterns of resistance, 2) to quantitate the frequency of development of resistance, 3) to establish means to prevent the appearance of resistance, 4) to find new techniques and tools, studying resistance, tissue culture methods, 5) to find new antagonists in the de novo systhesis of the purine ring.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 427(a)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$20,100	$6,630	$26,370	½	2	2½

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	2	2 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15.427(a)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Law, L. W. and Michael Potter: The Behavior in transplant of lymphocytic neoplasms arising from parental thymic grafts in irradiated, thymectomized hybrid mice. Proc. Nat. Acad. Science V42: 160-167, March 1956.

Potter, Michael and Lloyd W. Law: Studies of a Plasma Cell Neoplasm of the Mouse. I Characterization of neoplasm 70429, including its sensitivity to various antimetabolites with the rapid development of resistance to Azaserine, DON, and N-methyl Formamide. J. Nat. Cancer Institute. In press.

Potter, Michael, John L. Fahey, and Ira S. Pilgrim: Abnormal Serum Protein and Bone Destruction in a Transmissable Mouse Plasma Cell Neoplasm (Multiple Myeloma). Proc. Soc. Exptl. Biol. & Med. In press.

17. None
HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 427(b)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia Studies Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the etiology and chemotherapy of experimental lymphomas
PROJECT TITLE

7. Bernard Shacter
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Project: Biochemical mechanisms of resistance to metabolic antagonists in chemotherapy of experimental lymphomas.

Objectives:

The purpose of this project is to discover the biochemical mechanisms involved in the development of resistance of experimental lymphomas to chemotherapeutic agents.

(Use reverse and additional pages, if necessary)

Methods employed:

Searches are made for biochemical differences between experimental lymphomas resistant to chemotherapeutic agents as compared to parent lines of the same lymphomas sensitive to the agents. These include studies of possible differences in content of key metabolites and enzyme systems, as well as of differences in effect of chemotherapeutic agents on metabolic activities.

Major findings:

The fundamental biochemical differences between neoplastic cells sensitive to the growth inhibitory action of chemotherapeutic agents and cells resistant to such agents remain largely unknown. Hirschberg had found that several neoplasms sensitive to azaguanine were low in azaguanine deaminase while neoplasms resistant to the agent had higher levels of the enzyme. This suggested the possibility of resistance to azaguanine being related to higher levels of azaguanine deaminase in the neoplasms. The problem was investigated in this laboratory by comparing the azaguanine deaminase activities of several ascitic lymphocytic leukemias of mice. No distinct differences in enzymatic activity were apparent between leukemic lines susceptible to the growth inhibitory action of azaguanine, as compared to sublines of the neoplasms showing either derived resistance to or dependence on the agent.

The activity of choline oxidase, an enzyme occurring in the livers and kidneys of many species, is markedly inhibited in these organs following administration of folic acid antagonists. The enzyme occurs in chick bone marrow, a tissue profoundly affected by folic acid antagonists. Deprivation of folic acid or administration of antagonists leads to complete disappearance of the enzyme in this tissue. On the basis of these earlier observations it was considered of interest to investigate the possible occurrence of choline oxidase in experimental leukemias sensitive to folic acid antagonists.

The enzyme occurs in ascitic cells of lymphocytic leukemia L4946, a neoplasm profoundly affected by amethopterin. Administration of amethopterin to mice bearing well advanced growths of the neoplasm results in marked decreases in numbers of neoplastic cells as well as in concentration of choline oxidase. The significance of this finding is difficult to assess at present, since an opposite effect, increase in enzyme concentration, occurs during growth of the neoplasm. Of interest is the observation of remissions following administration of amethopterin to mice bearing ascitic growths of L4946 during the very late stage of proliferation.

Significance to cancer research:

The observation that suppression of growth of an experimental lymphocytic leukemia by amethopterin is accompanied by decreased activity of an enzyme system which may be intimately involved in the growth process, points to a possible mechanism of action of the antimetabolite. Moreover, it opens possibilities for utilization of other chemotherapeutic agents in treatment of lymphocytic leukemias; for if folic acid antagonists do indeed inhibit leukemic growth by inhibiting choline oxidase, then other inhibitors of the enzyme may also be effective.

Proposed course of project:

It is proposed to continue the investigations along the lines already started. The significance of occurrence of choline oxidase in leukemic cells as well as of inhibition of the enzyme by folic acid antagonists will be studied further. The effect of other inhibitors of choline oxidase, such as 2-amino-2-methyl-propanol-1, on growth of the neoplasms will also be investigated. If time permits, searches will be made for other key enzyme systems affected by folic acid antagonists which may occur in leukemic cells.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 427(b)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$16,080	$5,310	$21,390	1	1	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	0

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. 427(b)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Shacter, B.: Interrelations in respiratory, phosphorylative, and mitotic activity of Ehrlich ascites-tumor cells: Influence of stilbestrol and quinone. J. Nat. Cancer Inst. 16: 1453-1458, 1956.

Shacter, B, and Law, L. W.: Influence of amethopterin on tumor growth and liver glutathione levels of mice bearing lymphocytic leukemias. J. Nat. Cancer Inst. 17: 391-398, 1956.

Shacter, B., and Law, L. W.: Azaguanine deaminase activity of several lymphocytic leukemias of mice. J. Nat. Cancer Inst. In press.

Shacter, B.: Choline oxidase activity of L4946 leukemic cells of mice. J. Nat. Cancer Inst. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

No entries

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 427(c)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia Studies Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on Biochemical Mechanisms of Sensitivity and Resistance to Antimetabolites
PROJECT TITLE

7. Elizabeth P. Anderson
PRINCIPAL INVESTIGATOR

8. (see text)
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

A. Studies on Azauracil.

The objectives of this problem have been to investigate biochemical effects of 6-azauracil, with particular reference to effects on galactose metabolism. Studies by Handschumacher and Welch on an azauracil-resistant strain of Lactobacillus bulgaricus had indicated that this resistance could be maintained on glucose but not on galactose. Uridine nucleotide coenzymes are known to be necessary for the metabolism of galactose-1-phosphate, and an azauracil analog of these nucleotides seemed a possibility.

(Use reverse and additional pages, if necessary)

The work has had two phases. In one series of experiments azauracil has been administered to experimental animals and to terminal human leukemic patients, in the latter case as chemotherapeutic treatment. This work has been correlated with the program of Dr. Charles Gubrod and various members of his staff in NCI, particularly Doctors Emil Frei, David Rall, and Bernard Landau. My own study has been an examination of blood, drawn before and during azauracil administration, for enzymatic metabolism of galactose-1-phosphate. The method worked out has been an assay for the incorporation of radioactive galactose phosphate into a nucleotide form.

The other phase of the work has been comprised of experiments on the effect of azauracil and possible nucleotide derivatives of it on crude and purified forms of enzymes involved in galactose metabolism. The assays have been largely spectrophotometric and have drawn on earlier work carried out in the laboratory of NIAMD with which I was previously associated.

The analysis of blood of leukemic patients receiving azauracil did not indicate a decrease in the incorporation of galactose phosphate, compared with that before drug administration, except in one instance (total of five patients studied over a period of one to five months); this, however, was a progressive and marked decrease to 20% of the "normal" value at a time shortly before death. There was considerable variation from one individual to another in the levels of enzymatic activity reflected in the assay, and a further complication in the interpretation of the values was the element of transfusion. In two experiments on dogs, the administration of azauracil, at much larger doses, but for shorter periods of time, produced no significant change in the enzymatic metabolism of galactose phosphate in the blood, as indicated by this assay. At the present time it cannot be concluded that the marked decrease in ability to metabolize galactose observed in the one human case bore any relation to drug administration; it may have simply reflected a fall in enzyme activity in a very terminal case.

Experiments have detected no inhibitory effect of azauracil on galactose-1-phosphate uridyl transferase (which converts galactose-1-phosphate into uridinediphospho-galactose), either in crude tissue preparations or in purified form; likewise no effect is exerted on purified uridinediphospho-glucose dehydrogenase. Nucleotide fractions have also been prepared from fresh post-mortem liver of one dog and two humans who had received azauracil. These fractions, which presumably would have contained any azauracil nucleotide formed, likewise produced no inhibition of galactose phosphate uridyl transferase or of uridinediphospho-glucose dehydrogenase, both of which had been purified from calf liver.

It should be pointed out that the assay used to determine galactose phosphate uridyl transferase does not eliminate the possibility that the sugar phosphate was incorporated into an azauridine nucleotide form, perhaps one non-functional in enzymatic reactions beyond this stage. However, an effect on the transferase seems to be fairly completely ruled out, and any azauridinediphospho-glucose should have been detected in the assay and was not. In the experiments with purified enzymes the species difference could have been significant, but the results would seem to be even stronger evidence against the existence of azauridine nucleotides.

It is concluded that no evidence for azauridine nucleotide coenzymes has been found and no effect on galactose metabolism demonstrated by these studies. Some additional studies on liver enzymatic activity are in progress, but beyond that it is not planned to carry the problem further.

In addition to collaboration mentioned, acknowledgement is also due to Dr. H. M. Kalckar's laboratory, and to Dr. R. O. Brady's laboratory, for provision of some of the materials used in this study, and to both Dr. Kalckar and Dr. B. L. Horecker, NIAMD, for generous provision of working space and equipment until a laboratory in NCI could be provided and equipped.

B. Studies on Azaserine.

This is the first of a series of investigations planned on the metabolism of strains of leukemia, sensitive and resistant to antimetabolites. The objectives are to look for possible qualitative biochemical differences in the metabolism of such sensitive and resistant strains.

One biochemical action of azaserine has been defined in a competitive antagonism of glutamine. Thus azaserine has been found to inhibit various glutamine-requiring reactions, specifically the amination of formyl-glycinamide ribotide to formyl-glycinamidine ribotide, the amination of phosphoribosylpyrophosphate to phosphoribosylamine, and the amination of xanthylic acid to guanylic acid; of these the first is the most strongly inhibited.

The present work has used azaserine-sensitive and -resistant strains of the plasma cell neoplasm 70429 described in another section of this report (Dr. M. Potter). The tumor was carried in ascitic form in C3H mice.

The biochemical work has been carried out in full collaboration with Dr. Bruce Levenberg, NIAMD. Cell-free extracts of the cells have been prepared and their activity for the conversion of formyl-glycinamide ribotide through formyl-glycinamidine ribotide to amino-imidazole ribotide (measured as diazotizable amine) has been tested. Inhibition of this conversion by azaserine has also been tested in this cell-free system.

It has been found that cell free preparations of both the sensitive and resistant strains of 70429 are like pigeon liver in being able to convert formyl-glycinamide ribotide to amino-imidazole ribotide as well as to carry out the further conversion of amino-imidazole ribotide to amino-imidazolecarboxamide ribotide. Accumulation of these intermediates was sufficient to allow measurement as diazotizable amine. It was also found that the conversion was inhibited by azaserine and that the sensitive and resistant strains were qualitatively alike in the inhibition by azaserine.

This investigation is being pursued further with this and related approaches in the attempt to discover and define the biochemical similarities and differences between these two strains.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 427(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$4,030	$1,320	$5,350	½	0	½

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	0	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 427(d)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia Studies Section
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the etiology and chemotherapy of experimental lymphomas.
PROJECT TITLE

7. S. E. Reaume
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

Investigate genetic and biochemical mechanisms involved in the development of drug-resistance and -dependence in mouse neoplasms.

Methods employed:

1) Model bacterial systems have been employed for genetic studies of the frequency and mode of development of resistance to some glutamine analogs which are effective antineoplastic drugs.

2) Genetic analysis of a group of azaserine-resistant mutants has been undertaken to determine the allelic status and linkage relationships of genes responsible for resistance.

(Use reverse and additional pages, if necessary)

3) In vitro studies with E. coli have been made to determine whether or not resistance to glutamine antagonists is accompanied by either qualitative or quantitative changes in the biochemical pathway of de novo purine synthesis.

4) An attempt has been made to select leukemic lines resistant to the action of the purine analog, 4-aminopyrazolo-(3,4-d)-pyrimidine(APP).

Major findings:

1) In an azaserine-sensitive strain of the bacterium Escherichia coli, K-12 , 2 different resistant phenotypes occur; a "high-resistance" type which is unaffected by azaserine at near-saturation levels; and a "low-resistance" type which is inhibited at levels of about 200 gamma/ml, but not below that level (an approximate 20-fold increase of resistance over the parental tolerance). Both mutations appear to be of the "single-step" type. Reversions have not been observed.

2) "High-resistance" types of E. coli show some concomitant resistance increase to unrelated antibiotics (streptomycin, penicillin) which suggests resistance may be due to membrane permeability changes. These strains are also infertile, a fact which might be construed as consistent with cell surface modification. Infertility of this mutant type has, of course, precluded formal genetic analysis of the locus or loci involved. The "low resistance" mutant retains its fertility and resistance segregates as a single gene. Incomplete data suggest that the gene responsible for this type of resistance is located in the right arm of the chromosome (i.e., to the right of the E/Hfr/Gal complex), possibly near the Ara locus.

3) Attempts to obtain cell-free preparations of E. coli K-12 which synthesize purines de novo (Buchanan-Greenberg pathway) have thus far been unsuccessful.

4) Selection for resistance of leukemia L-1210 to APP has not been successful. The sensitive line, L-1210(S) has been selected for 27 transplant generations. Two sublines of the azaguanine-resistant leukemia, L-1210 (AgR), have been selected for 30 and 54 transplant generations, respectively. Both ascitic and solid tumors have been used. On several occassions resistance seemed to be obtained, as judged either by the large size of the solid tumor or the shortened survival time of an animal carrying the ascitic leukemia. In all cases, however, quantitative tests for resistance to APP gave only negative results. Studies of the ability of 4-hydroxy-6-aminopyrazolo-(3,4-d)-pyrimidine (HAPP) to substitute for 8-Azaguanine (8-Ag) for an 8-Ag-dependent tumor have had to be discontinued pending clarification of the structure of the compound originally used. It also appears possible that the activity of

interest resided in an impurity, the nature of which is unknown. These chemical problems are being studied by Dr. R. K. Robbins, New Mexico Highlands Univ., our source of supply of HAPP.

Significance to the program of NCI:

Presently available technics are inadequate to permit a direct, detailed attack on problems of the development of drug resistance in leukemic cells. It is hoped that studies of model mutational and enzymatic systems such as E. coli K-12 may shed some light on the genetic and biochemical events which accompany resistance to anti-neoplastic drugs. While any such information may not be precisely applicable to the situation obtaining in leukemias and similar neoplasms, the success of the application of principles of comparative biochemistry in the last 3 decades, and of biochemical genetics more recently, would appear to validate these studies in terms of cancer research.

Proposed course of project:

Quantitative studies of frequency of mutation to the 2 types of azaserine resistance are planned, as well as continuation of mapping and allelism tests of a number of independently-occurring resistant clones. It is of much interest to know whether resistance to azaserine can arise by mutation at more than one locus. Since azaserine inhibits, though weakly, other reactions than the amidation of formyl glycineamide ribotide, non-allelic mutations affecting other enzymatic steps might be expected. If they occurred, and a similar situation obtained in tumors, the probability of resistance occurring in a given cell population might be quite high. Such a state of affairs would impose perhaps severe limitations on the clinical application of this drug.

Although the de novo purine synthesis system has not as yet been obtained in vitro, methods of preparation of active extracts have hardly been exhausted and efforts along these lines will be continued in collaboration with Doctors E. P. Anderson (NCI) and Bruce Levenberg (NIAMD).

The extent to which work on APP-resistant leukemias will be pursued is uncertain. While a doubly-marked drug resistant leukemia would be of great interest, our inability to obtain any kind of stable resistance in over 50 transplant generations is discouraging. This fact alone, however, is curious. The inference could be drawn that mutation to resistance, if it occurs at all, is an extremely infrequent event. In this respect APP has an advantage over other anti-leukemic drugs to which resistance develops rather rapidly. Perhaps APP would have therapeutic value as an adjunct to other, more powerful inhibitors since APP resistance so seldom occurs.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 427(d)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$8,040	$2,640	$10,680	1	0	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	0	1	0

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, & 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 427(e)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia Studies Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. A study of the etiology and chemotherapy of experimental lymphomas
PROJECT TITLE

7. Lloyd W. Law and Delta E. Uphoff
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

This report deals primarily with the use of x-radiation and bone marrow as experimental variables in the study of leukemia. The projects may be classified under the following two major headings: 1) Etiology and pathogenesis of experimental leukemias; 2) Chemotherapy of transplantable leukemias.

Much of this work was started in the fall of 1954 and some was included in the 1954 reports of Dr. Law and Dr. Potter.

(Use reverse and additional pages, if necessary)

1) Etiology and pathogenesis of experimental leukemias.

A. The influence of thymectomy on the spontaneous incidence of leukemia in (C3HfxAKR)F_1 mice is being studied. Twenty male and female (c#HfxAKR)F_1 mice were thymectomized at about 5 weeks of age while 20 intact littermates were kept as controls. They are now 5 months of age with no deaths from leukemia in either group to date.

B. Study of "bone marrow repressive" factor in spontaneous lymphomas.

1. One hundred and eleven littermate (C3HfxAKR)F_1 mice were devided into 2 groups, one receiving an intravenous injection of C3Hf bone marrow while the other received AKR bone marrow. This was done in an attempt to define more clearly the protective effect (Lorenz) of C3Hf marrow against the spontaneous incidence of lymphomas. Further, to determine if the use of marrow from the high leukemic strain, AKR, would have the opposite effect, namely, to increase the incidence of lymphomas. This work is still in progress.

2. An extension of the study of the influence of C3Hf marrow was attempted using the high leukemic strain AKR as test animals. Sixty AKR mice 2-3 months of age were given an intravenous injection of C3Hf bone marrow. A second group of 23 animals 1 month of age was given C3Hf marrow while noninjected littermates were kept as controls. There appears to be no protection afforded by the C3Hf bone marrow, although tabulation of age at death and types of neoplasms is still incomplete.

3. Upon the assumption that the C3Hf bone marrow cells are not surviving sufficiently long in the AKR mice to afford protection, the following study was initiated. Fifty AKR mice were exposed to 150r x-irradiation for 4 exposures at 7 day intervals. An intravenous injection of C3Hf marrow was given to half the animals following the last exposure. The noninjected littermates served as controls. While only a few cases of leukemia have occurred, after about 5 months, they have been found only in animals receiving C3Hf bone marrow.

4. Kaplan has shown that exposure of C57BL mice to fractionated doses of x-radiation at 2-3 months of age produces a marked increase in the incidence of leukemia. C57BL/Ka mice were exposed to 400r x-irradiation within 24 hours of birth to determine if a single dose of radiation would be leukemogenic when given at an early age. The mortality is high for the first week's, post-irradiation, but a hundred mice reached weaning age.

When the first leukemias appeared at 4 months of age, a second group of 43 mice was exposed to 400r at 1 month of age. Fifty-nine mice were exposed to 100r and 57 were exposed to 25r at birth. Preliminary results show 400r at 1 month of age to be leukemogenic but the incidence is lower and the onset is later than in the case of 400r at birth. The lower doses of 100r and 25r appear to be non-leukemogenic or the age at onset will prove to be very much later than in the other groups.

5. Spleen shielding (Lorenz) and intravenous bone marrow (Kaplan) protects C57BL/Ka mice from leukemia if it is given during or following exposure to 225r x 4 q7. New born C57BL/Ka mice were exposed to 400r and given an intraperitoneal injection of C57BL/Ka marrow from adult donors in an attempt to alter the incidence of induced leukemia. Only 27 mice survived beyond weaning age. Although this group is very small, and it is too early to determine what effect, if any, the intraperitoneal bone marrow will have on the incidence of leukemia; leukemia does occur. If the incidence of leukemia is sufficiently high in the group exposed to 400r at 30 days of age, it may be possible to alter this incidence by intravenous bone marrow injection. Another possible alternative is to expose the mice to 400r at birth and give the marrow by intravenous injection at 1 month of age.

6. The influence of bone marrow injection on methylcholanthrene induced reticular neoplasms is being studied. DBAf/2 mice were painted three times a week with a 0.2% methylcholanthrene suspension in either. One group of 48 mice received a total of 10 paintings while a second group received a total of 20 paintings. Half of the mice in each group received 2 intravenous injections of DBA/2 marrow on the second and 16th day after the last painting. A classification of the reticular neoplasms will be made when the experiment is completed.

2) Chemotherapy of transplantable leukemias.

A. New transplantable reticular neoplasms, both induced and spontaneous, are being developed. A granulocytic leukemia (H5530) arose spontaniously in a C58 female. The ascites form of this tumor has proved resistant to treatment with drugs commonly used on other tumors. The most promising results have been obtained (with Dr. Potter) by use of drug combinations. This work is still in its preliminary stages.

B. Preliminary work is underway on the treatment of drug toxicity by intermittant bone marrow injections. It was hoped that by periodic bone marrow injections there would be reseeding of the depleted marrow enabling the administration of larger doses of drugs. Although this work is still in the pilot study stage, some progress has been made. It was found that the mean survival time of AKR mice previously inoculated with L4946 was markedly increased when a combination of drug and bone marrow treatment was used. The mean survival times for the four groups in days were; 8 days for L4946 untreated; 18 days for L4946 + methotrexate; 25 days for L4946 + methotrexate + C3Hf marrow after each third injection (a total of 2 marrow injections); and 27 days for L4946 + methotrexate + AKR marrow (as above). Further work is in progress on the effectiveness of this treatment on normal and tumor bearing mice.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 427 (e)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$6,410	$2,110	$8,520	1/3	½	5/6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/2	5/6	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, & 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 427(f)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia Studies Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Genetics of radiation protection by bone marrow injection
PROJECT TITLE

7. Delta E. Uphoff
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

The ability to inoculate bone marrow between certain species and strains for protection against radiation mortality has passed an interesting genetic problem. An extensive survey was started with 2 main objectives; 1) to correlate, if possible, the ability to protect against radiation mortality with the histocompatibility types of the marrow donor and the recipient; 2) to determine if there is a relationship between protection against radiation mortality, thymic degeneration and regeneration and lymphoma induction. In this survey over twenty-five hundred animals have been used in 74 different combinations. (The broad scope of this work has been made possible through a generous supply of mice of different inbred strains provided by Dr. Margaret Deringer). Several interesting

(Use reverse and additional pages, if necessary)

observations stemming from this work are discussed below.

A. The cellular vs. humoral theory of radiation protection.

When this work was started there was little evidence in favor of the "cellular theory" of protection with many investigators favoring the "humoral theory". Cole has since shown that after protection with rat marrow the blood of the mouse shows an alkaline phosphatase activity characteristic of circulating rat cells. Ford using mouse bone marrow marked with a translocation (T-6) has demonstrated cytologically that tissues marked with this translocation survive for as long as 7 weeks post-irradiation. It can now be demonstrated by tumor transplantation that the bone marrow cells of the donor live as long as 10½ months post-irradiation. All animals surviving the acute post-irradiation stage have been observed for longevity and tumor incidence. To date, three lymphomas have been observed, and have been tested by transplantation to determine if they are of donor or recipient origin. The results are as follows:

1. (C3HfxAKR)F_1 female received 800r + intravenous AKR bone marrow. Seven months after treatment it developed a leukemia which did grow progressively following transplantation into both F_1 and AKR mice, indicating the leukemia was of AKR origin.

2. (C57BL/KaxA)F_1 male received 800r + intravenous AKR bone marrow. Six and a half months after treatment it developed a thymic leukemia which was transplantable back only to AKR mice; thus, again showing the leukemia to be of donor origin.

3. Lastly C3Hf female received 800r + intravenous C58 bone marrow and was shown to have developed, a C58 leukemia 10½ months after treatment.

An attempt will be made to produce these chimeras in larger numbers so that the biological mechanisms involved in radiation protection and tumor development may be studied.

B. The secondary post-irradiation syndrome.

In the early radiation protection work with Dr. Lorenz all experiments were terminated at 30 days or shortly thereafter. The important post-irradiation period beyond this short interval has been of great interest. It has been shown by many investigators that there is complete and lasting recovery if the animals receive isologous bone marrow (marrow from the same strain). Those mice receiving homologous marrow (that of another strain) usually recover by 14 days, the time at which all non-treated controls have succumbed; however, in most cases it is not a lasting recovery.

The onset of the secondary phase depends upon the strain of the recipient and the strain of the donor. It may start as early as 18 days or later than 40 days post-irradiation. This secondary syndrome is marked by diarrhea, weight loss, but normal food intake, degeneration and necrosis of lymphoid tissue, lack of hair-greying, occasional dermatitis, and occasional liver lesions (non-bacterial). A bone marrow has completely regenerated. After a long illness most animals succumb, but a few do survive the secondary phase; e.g. the animals which later developed leukemia as listed in section A.

The secondary phase has become the focal point of much work upon its causation and possible treatment (or prevention). The experimental criteria for the production of this secondary phase have not yet been fully defined except to the extent that it occurs when homologous marrow is used as a protective agent following a lethal dose of radiation. One exception to this is that marrow from the 2 inbred strains, BALB/c and DBA/2, may be used interchangeably in either animal as a recipient without any ill effects. The secondary syndrome also occurs when either one of the parent strains is used for protection in an F_1 hybrid, but not when the F_1 (isologous) marrow is used, see below. This finding has been demonstrated to be true for the following 3 hybrids; (C3HfxAKR)F_1, (C57BLxDBA)F_1 and (C57BLxA)F_1. As is the case with the inbred strains of BALB/c and DBA/2, either parent marrow protects as well as does the F_1 marrow when (BALB/c x DBA/2)F_1 mice are used as recipients.

In an attempt to determine the relationship of this secondary phase to the histocompatibility types of the donor and recipient, the isogenic resistant lines of C57BL/10 have been used. In the isogenic resistant lines, the animals are isogenic except for a difference of one histocompatibility gene at the H-2 locus (one designated as H-2b, the other as H-2d). Although the number of animals treated is still small, this secondary syndrome is observed when there is this specific one gene difference between the donor and the recipient. Furthermore, when the "hybrid" (C57BL/10 H-2d x C57BL/10 H-2b)F_1 is used as a recipient, there occurs the secondary syndrome (sometimes called the "F_1 effect"), when C57BL/10 H-2d marrow is used, but not when F_1 marrow is used. The second parent (C57BL/10 H-2b) has not as yet been tested in this "hybrid". This phase of the work indicates the importance of the H-2 locus; however, it must be confirmed before a true evaluation of these data can be made.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 427(f)
SERIAL NUMBER

12. BUDGET DATA:

ESTIMATED OBLIGATIONS			MAN YEARS		
DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
$9,380	$3,100	$12,480			
FY'57			2/3	½	1-1/6

BUDGETED POSITIONS			PATIENT DAYS
PROF	OTHER	TOTAL	
5/6	2 1/3	2 1/6	0
FY'57			

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, & 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 428
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Tissue Culture Section
SECTION OR SERVICE

5. Bethesda, Maryland
LOCATION (IF OTHER THAN BETHESDA)

6. Studies of cell nutrition in vitro.
PROJECT TITLE

7. Study C - Evans, V. J. and Sanford, K. K.
Study G - Evans, V. J. and Menefee, M.
All others - Evans, V. J.
PRINCIPAL INVESTIGATOR

8. Westfall, B. B. Fioramonti, M.C. Hawkins, N. M.
Bryant, J. C. McQuilkin, W.T. Earle, W. R.

OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS): Study A. Title: Development of a defined medium for the cultivation of clone strain 929 mouse fibroblasts in vitro.

Objective: To develop a satisfactory chemically defined medium adequate for producing proliferation of clone 929 mouse fibroblasts in order to study their nutritional and metabolic behavior.

Methods employed: Replicate cultures are prepared and enumeration of nuclei is made after exposure to defined media for varying intervals. Such media were devised from the collected information on the nutrition of bacteria, mammalian and avian cells in vitro and the data from physical and chemical fractionations of naturally occurring medium on which the strain 929 cells had been maintained.

(Use reverse and additional pages, if necessary)

Major Findings: A medium designated NCTC 109 has been devised which allows a continued reasonably rapid rate of proliferation for clone 929 cells without any protein additive. The essential amino acids have been determined and studies on the effect of the so-called non-essential amino acids on proliferation have been made. In the presence of serum the solution has been found to substitute entirely for embryo extract for proliferation of all cell strains studied to date.

Significance: This particular medium has considerable significance because so far as is known it is the only completely chemically defined protein-free medium which allows continuous maintenance and reasonably rapid proliferation of any strain of tissue in vitro. Such a chemically defined medium is essential for determining differences in the metabolism of normal and malignant cells and for research in the nature and origin of the malignant transformation. This medium is invaluable as a test medium to determine whether or not other cell types can be cultured in it. Tissue cultured cells grown in it with and without serum supplementation become valuable tools for studies in chemotherapy virology, carcinogenesis and many related fields.

Projected Studies: Continued research will be done to determine the essentiality of the groups of components as well as the individual members of the group and imminently to determine the function of each of the "non-essential amino acids" on proliferation. Fortification studies of this medium are also planned. Metabolic studies are in progress on several cell strains in this medium.

Study B. Title: Evaluation of NCTC-109 in long term cultivation of NCTC Clone 929 cells and a study of the process of the adaptation of the cells.

Objective: To continue the evaluation of the completely defined medium NCTC-109 regarding its ability to maintain NCTC Clone 929 of mouse fibroblasts in a reasonably rapid rate of proliferation for an indefinite period. To study the process of adaptation of the cells to this new protein-free medium.

Methods employed: Fifty-seven attempts have been made to adapt separate cell lines of NCTC Clone 929 fibroblasts to continued maintenance in protein-free medium NCTC 109. The cells had previously been carried in EE-HS saline medium or the NCTC 109 supplemented with 1/4% HS. All cultures were grown in stationary flasks and have generally been treated in routine long term tissue culture manner with serial transfers being made as growth rate warranted. Both written and photomicrographic records were maintained to follow changes in cell morphology and growth rate.

Major findings: 1) Of the 57 attempts to adapt separate lines of NCTC Clone 929 cells to NCTC-109 only 10 have been successful. 2) In changing from a protein medium to the protein-free medium, the following points appear to stand out: a) a morphological change does take place during the adaptive process, b) the cells undergo a critical period during which they either succumb or adjust to the new mileu, c) the cells that do adapt apparently undergo a functional change which enable them to survive and proliferate in a nutritional medium foreign to their accustomed one, d) once a culture has adapted to the new medium its vitality and ease of maintenance appears to equal that of the parent cell strain which was maintained in protein containing solution, e) cell populations do not become as great in the chemically-defined medium as in the biologic medium. f) there appears to be a difference in the cell surface of adapted cells that causes them to attach to the glass substrate tighter than the cells formerly did when in the protein medium.

Significance: This study is part of the evaluation of the NCTC-109, which is the only chemically-defined medium that has been used successfully to maintain cells indefinitely in a proliferating state. Although it proves that the medium can maintain the cells indefinitely, it also has shown that as the medium is presently constituted the cells or cell population must undergo some morphological and functional changes in adapting to the medium.

Proposed course or project: Comparison between the parent cell strain on protein medium and a NCTC-109 adapted line with the use of time lapse microcinematography on mitotic rates and intervals, and on rate, form, and nature of cell migration are planned.

Study C. Title: A study of the effect on Clone 929 mouse fibroblasts of NCTC 109 chemically defined medium as compared with the effect of the same medium when 13 presumably non-essential amino and amides are omitted.

Objective: To determine the effect of omitting 13 "non-essential" amino acids, amides and amines from the previously formulated chemically defined nutrient solution designated as NCTC-109 in long term cultivation of NCTC strain 2071 cells. (This strain of cells is a subline of clone 929, and has been maintained in this NCTC-109 protein-free solution for 1 3/4 years to date.)

Method employed: Two identical solutions of NCTC-109 were made up, with the exception that in the experimental solution 13 "non-essential" amino acids were omitted, and of the 13 "essential" amino acids used, 4 were tripled in concentra-

tion. Five separate lines of 2071 cultures were maintained by long term procedures with serial transfers on each of the two media. Photomicrographic records were maintained. Short term replicate culture experiments were also conducted to compare rate of growth quantitatively.

Major findings: Of the 26 amino acids, amines, and amides of the protein-free medium, those essential for proliferation of strain L cells were determined. From dose-response curves, new mixtures were devised. In a long-term 4 month growth study, the mixes of amino acids previously considered essential failed to give the degree of proliferation obtained with the original mixture of 26 even when amino acid nitrogen levels were comparable.

Significance: A simplified chemically defined medium is of primary significance in order to determine differences that may exist in the nutritional requirements of malignant cells in vitro as compared with the normal cells from which they arise.

Projected studies: To conduct further studies to determine the relative importance of each of the amino acids, amines and amides studied.

Study D. Title: Study of the use of metabolite and antimetabolite systems in tissue culture.

Objective: To determine the growth promoting effect of biotin on strain 2071 and strain 2168 cells of Clone 929 strain mouse fibroblasts in a chemically defined, protein-free medium. (These two strains had previously been maintained in NCTC 109 and in NCTC-109 and $\frac{1}{4}$ percent horse serum respectively.)

Methods employed: Replicate cultures were prepared and nuclei were enumerated to determine the change in cell population level from the inoculum population in chemically defined protein-free media containing biotin in the presence and absence of the antimetabolite avidin, and in media from which biotin has been omitted in the presence and absence of avidin.

Major findings: Biotin is a growth promoting factor for cells of these strains in chemically defined protein-free medium NCTC 109 and 110.

Significance: The determination of biotin as a growth promoting factor for the strain 2071 and 2168 cells and the utilization of an antimetabolite in tissue culture techniques.

Projected studies: To determine more specifically the nutritional and metabolic needs of tissue culture cells in a

protein-free chemically defined medium. To determine whether or not the metabolite-antimetabolite system can be used to determine other "growth promoters", and determine whether the medium NCTC 109 and 110 can be further improved.

Study E. Title: Adaptation of mouse liver cells to cultivation in a protein-free chemically defined medium.

Objective: To adapt mouse liver epithelial cells to cultivation in protein-free chemically defined medium in order to study their nutritional and metabolic behavior.

Method employed: NCTC Clone 1469 and Strain 1795 (of low and high tumor producing incidence respectively were subjected to gradual reduction of the normal serum component of their medium. As this reached progressively lower levels and the cells began to lose their ability to adhere to the glass floor of the flask, they were transferred to the roller tube type of cultivation in order to avoid critical loss of population at fluid renewals. At intervals determined by the condition of the cells, serum volume was further decreased until cells were grown in the chemically defined medium NCTC 109 to which no serum has been added. Progressive subculturing was continued to dilute out all effects of protein originally in the medium.

Major findings: To date the medium on these cultures has been reduced to $\frac{1}{4}$ percent by volume of serum. At present the cells are free floating and satisfactorily proliferating in fluid suspension. The use of the roller tube is essential in order to retain population initially since the cells in early stages of adaptation float in suspension. It further appears that maintenance of a certain minimal cell population density is particularly critical during this process of adaptation, however. Once the cells have become adapted in adequate numbers they proliferate and can be carried as an adapted strain in NCTC 109 without protein.

Significance: It is of significance to demonstrate nutritional differences between the two strains of liver cells, and to define other factors relating to their growth.

Projected studies: Continued effort will be made to adapt these strains to medium containing no protein. It is further proposed to continue study of nutritional differences between the lines of high and low tumor incidence and to test any a available purified protein fractions or substitutes.

Study F. Title: Cultivation of mouse and human fresh tissue *in vitro* in chemically defined medium.

Objective: To cultivate fresh tissues explants from human infant and C3H mice directly in chemically defined medium NCTC 109 without the contaminating effect of any heterologous proteinic materials.

Methods employed: Fresh tissu were removed aseptically and planted under cellophane in NCTC-109. Connective tissue, heart, muscle, liver, spleen, kidney, pancreas, and lung have been used.

Major findings: Cell outgrowth from these freshly planted tissues has been obtained only with mouse connective tissue and lung tissue. Best results have been obtained with lung tissue; viability has been maintained for over 130 days.

Significance: This is an attempt to establish cell strains adapted to chemically defined medium without the cells having undergone any possible alterations from a heterologous proteinic medium. Although cell strains have not been successfully established to date, the long periods of vaiability so far attained give reason to believe that success can be achieved when certain technical difficulties such as the adverse influence of blood cell debris can be eliminated. It is a question of major significance as to whether cell strains established in this way will ultimately undergo spontaneous malignant alteration.

Projected studies: To continue in the effort to establish cell strains of fresh tissue. In this manner, and to determine whether such cell strains do undergo spontaneous malignant alteration.

Study G. Title: Electron Microscopy studies on Clone 929 strain of mouse fibroblasts and clone strains of human skin epithelial cells to determine variations in cell structure in 3 types of media.

Objective: A study of the changes occurring in cells on three types of media and how these cells appear when observed as sections in the electron microscope.

Methods employed: The usual methods employed in electron microscopy were Dalton's or Palade's fixative (Buffered Osmic) and the special technique of post osmication in 2% unbuffered osmic acid which demonstrates reducing materials such as unsaturated lipid. Sections are made of the fixed cells. Tissue cultured cells of mouse fibroblasts and human skin epithelium on high and low serum protein medium, and on protein-free chemically defined medium NCTC 109 were used in the study.

Major findings: Differences that occur were between cultures growing on any protein and no protein. These differences appeared in mitochondria, lamellated bodies derived from mitochondria, and other possible effects derived from mitochondrial activity such as deficiency in the cytoskelton. Ordinarily there is no reducing material in mitochondria of normal animal cells or cells grown on protein containing material. In the protein-free media the mitochondria provide a great amount of reducing material and no lamellar mitochondrial derivatives were observed; the cytoskelton was considerably more fragile.

Significance: There appears to be some difference in the protein-free medium which results in alterations in the protein synthesis.

Projected studies: To examine both strains of cells after alterations of amino acids and vitamin contents of the medium.

Study H. Title: Adaptation of human skin epithelial cells to cultivation in a protein-free, chemically defined media.

Objective: To determine the essential amino acids and other non-protein derivatives necessary for the long-term, large-scale production of human skin epithelial cells. To adapt and maintain in long-term, large scale tissue culture a strain of the human skin cells for study of possibility of application on the critically burned or otherwise denuded patient.

Methods employed: Fluid stock cultures of each of the cloned human skin strain (NCTC Clone 2271 and 2414) cultivated on a dilute human serum and chick embryo extract mixture, was removed and replaced with a media composed of NCTC 109 and human serum, 90 and 10% respectively. After 7 days in culture with 3 fluid changes the media was replaced with a more dilute serum mixture (5%), followed by a further dilution to $2\frac{1}{2}$, 1 and 0 percent respectively, at 14, 21 and 28 days respectively.

Major findings: At the end of 30 days, when the residual serum level was less than 1%, it was observed that the cells of each culture no longer adhered to the glass floor of the flask, but were maintained free floating in the nutrient fluid. The cells were then transferred to, and maintained in, rotating roller tubes. While nuclear enumeration was not done, a continual shift in pH and obvious increase in cell population,

indicated that the cells were viable; proliferation was not so rapid that transfer to daughter roller tube cultures was necessary at approximately every 7 to 10 days.

Significance: An adaptation of the human skin cells to a completely chemically defined media in massive culture would be potentially significant as covering of denuded areas. Such an adaptation will eliminate serum as source of contaminating viruses. It is also quite possible that the elimination of homologous and heterologous protein might reduce the possibility of a malignant transformation in vitro.

Projected studies: To eliminate by qualitative and quantitative methods, all unnecessary components of the present chemically defined protein-free culture medium NCTC 109 for the growth of the human skin epithelial strain. To study the cells so grown on a chemically defined medium in further transplantation studies.

Study I. Title: Adaptation of established strains of cells to media of heterologous composition.

Objective: To adapt a normal and a malignant strain of cells to media of complete heterologous composition: a) To evaluate a possible transformation of the normal strain to a malignant strain, and b) to evaluate for its possible virological application a human cell in a media void of any homologous virus antibody.

Methods employed: The method employed in adapting strains of cells has been described in previous annual reports.

Major findings: The human skin NCTC Strain 1769 has been adapted to cultivation on a completely heterologous media for approximately 1 year and is designated as NCTC Strain 2198. The human cervical carcinoma HeLa NCTC Strain 1881 has been adapted to cultivation on a completely heterologous media for approximately 3 years and is designated NCTC Strain 1985.

Significance: No striking morphological transformation were observed during this adaptation; however, the adaptation to the new medium was successfully carried out.

Projected studies: No extensive continuation.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 428
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$28,120	$9,280	$37,400	1	2 1/2	3 1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	2 1/2	3 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S): Section of Cellular Biology, Laboratory of Biology, NCI

Tissue Bank, National Naval Medical Center.

(Use reverse and additional pages, if necessary)

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Studies of nutrient media for tissue cells in vitro. I. A protein-free chemically defined medium for cultivation of strain L cells. Virginia J. Evans, Jay C. Bryant, Mary C. Fioramonti, William T. McQuilkin, Katherine K. Sanford, and Wilton R. Earle. Cancer Research, 16: 77-86, 1956.

Studies of nutrient media for tissue cells in vitro. II. An improved protein-free chemically defined medium for long-term cultivation of strain L-929 cells. Virginia J. Evans, Jay C. Bryant, William T. McQuilkin, Mary C. Fioramonti, Katherine K. Sanford, Benton B. Westfall, and Wilton R. Earle. Cancer Research, 16: 87-94, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 429
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Tissue Culture
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Studies of the influence of cell population on proliferation.
PROJECT TITLE

7. (a) Sanford, K. K.
(b) Earle, W. R. and Bryant, J. C.
PRINCIPAL INVESTIGATOR

8. (a) Hobbs, G.L., Schilling, E. L., Earle, W. R.
Evans, V. J.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS): Study A. Title: Single cell studies and the development of cell clones.

Objective: To develop clones of mouse mammary carcinoma, mouse sarcom and mouse normal tissue, and to more accurately define and control factors allowing the growth of single isolated tissue cells.

Methods employed: A sieved cell suspension is prepared and drawn into capillary pipettes. Segments of the capillary are cut so as to contain one isolated cell. The segments are attached to the floor of the Carrel flask by a thin plasma film, and the culture fluid is renewed thrice weekly. The culture fluid is NCTC-109 supplemented with 10% horse serum.

(Use reverse and additional pages, if necessary)

Major findings: It has been found that many cell strains cannot easily be returned to chicken plasma after growth on glass or cellophane, except after readaptation. For this reason many of our earlier attempts to establish single cells failed. It has also been found that chemically defined culture medium NCTC-109 supplemented with 10% serum promotes single cell growth far better than the serum-chick embryo extract culture fluid used earlier. By application of these findings, establishment of clone strains has been greatly simplified, so that more than 12 clone strains of various cell types under study have recently been established.

Significance: The development of clones of cells is of fundamental importance for certain problems in carcinogenic cell transformations among populations of cells in vitro, virus studies, and in studies of the differences in the physiology and nutrition of malignant cells and the normal cells from which they arise in culture. The method now in use is in our opinion the most precise and reliable for originating strains assuredly of single cell origin.

Projected studies: There will be further work in attempts to define factors allowing reliable growth of isolated cells. Efforts will also be made to use principles developed in reliable growth of clone strains from fresh tissue. These developments, however, will be of secondary activity since procedures now routinized give us as many clone strains as we now can use efficiently in our area of research. Actual use of these various clone strains will be considered under other projects.

Study B. Title: Growth of massive fluid suspension cultures of animal tissue cells.

Objective: Growth of massive fluid suspension cultures of both normal and malignant animal tissue cells. To develop methods and equipment for growing massive fluid suspension cultures of animal tissue cells. To define factors involved so that the growth of desired cell types can be reliably and accurately routinized, and so that growth of cultures of other sizes can be reliably extrapolated.

Methods employed: The standard New Brunswick type platform shaker inclosed in an incubator box, as previously developed was used for continuing studies with massive agitated suspension cultures. The shaker was operated at 8600 revolutions per hour instead of the 12,000 rph rate used previously. Culture flasks were 1½ liter in size, containing a maximum of 500 ml. of cell suspensions. Continuous aseptic flow of 5% CO_2 in air, or in 40% oxygen saturated with water vapor, was maintained through each culture flask. The rate of gas flow

was held constant at either 140 or 280 ml. per hour. The pH indicator was phenol red at a concentration of 0.002%, in all of the culture fluid. The fluid was renewed periodically without loss of cells. The rate of proliferation of cells in suspension was determined at every fluid change, thrice weekly, by nuclei counts on accurately measured aliquots. A short modification of the standard Evans-Sanford method for counting nuclei was adopted in order to permit estimation of the cell population immediately before each fluid change.

Major Findings: Examination of the major factors governing the growth of cell suspension cultures in shaker flasks has been continued, and considerable progress has been made in developing the method for reliable routine growth of large amounts of tissue cells. The effect of various levels of glucose was studied in considerable detail, and are reported under a separate project. Various media used included either a whole egg ultrafiltrate, or chemically defined medium, with low concentration admixtures of serum. Control of pH has been improved over that previously attained. Continued renewal of a CO_2 - enriched atmosphere over the culture fluid, and the inclusion of phenol red at a level of .002 percent in the medium, permitted control of pH within the limits which have been found to be optimum for satisfactory cell proliferation i.e., pH 6.8 to 7.5. In certain instances where there appeared to have been an insufficient level of CO_2 in the flowing air, the pH of the fluid increased slightly during the fluid change interval from 7.4 to 7.6 while the population increase was distinctly retarded; the normal trend of pH in these agitated cell suspensions during a 2 or 3-day fluid change interval in the logarithmic phase of growth, was from pH 7.35 to about 7.1.

The use of protein-free chemically defined medium NCTC-109 as a complete medium for various strains of cells was attempted, without results for any strain. The addition of some protective material associated with serum protein was found necessary as a supplement to the protein-free chemically defined medium NCTC 109, although such supplementation is unnecessary with a number of cell strains in unagitated T-60 flask cultures. It has not been determined whether the major effect of serum is protective or nutritive, or both. The degree of cell proliferation was positively correlated with the proportion of serum used, within the range of 20, 15, and 10% by volume.

Maintenance of a uniform cell population density at each fluid change appears to be only one of several factors influencing the proliferation of cell suspensions in complex patterns of interaction.

The number of established tissue cell strains successfully grown in these massive cultures was increased during the year from five to seven.

Significance: The project is of major significance to cancer research, immunology, metabolic studies, and many related fields in making available methods by which large amounts of cells of pure strains, can be grown. Results indicate that the size of such cultures can be increased many fold as better control of factors involved is achieved.

Projected Studies: Further work is being pressed in order to more accurately define and isolate the variables governing proliferation and certain aspects of metabolism. Agitating mechanisms other than the New Brunswick type shaker are in process of development for more accurately defining factors and rates of growth.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 429
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$29,450	$9,700	$39,150	2 1/3	1 1/3	3 2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2 1/3	1 1/3	3 2/3	0

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Growth of cell suspensions in tissue culture. Wilton R. Earle, Jay C. Bryant, Edward L. Schilling, and Virginia J. Evans. Annals of the N.Y. Acad. of Sc., 63: 666-682, 1956.

LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 430
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Tissue Culture Section
SECTION OR SERVICE

5. Bethesda, Maryland.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the cultivation and function of epithelial and secretory cells.
PROJECT TITLE

7. Evans, V. J.
PRINCIPAL INVESTIGATOR

8. Hawkins, N. H., and Earle, W.R.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS): Study A. Title: A study of the extent of differentiation occurring in new born rat ovaries in vitro.

Objective: To study the extent of differentiation occurring when new born rat ovaries were cultured for varying periods of time under different culture conditions.

Methods employed: New born rat ovaries, both intact, and divided, were cultured in vitro in thin plasma clots and in a variety of media and with variations in the amount of oxygen supplied constantly to the cultures.

(Use reverse and additional pages, if necessary)

Major findings: The tissues were maintained in excellent condition from treatment with the protein-free chemically defined medium NCTC 109. Apparently the small amount of plasma required to keep ovaries clotted to the flask floor served as adequate supplement for the chemically defined medium. No differentiation beyond that seen in rats 7 days in vivo was obtained irrespective of the fact that one series of cultures was maintained 6 weeks in vitro. The best differentiation of 7 days was obtained with 60% oxygen tension and the CDM.

Significance: Any endocrine tissue cultivable in vitro with the accompanying data obtained could serve as indices of changes in cells in vitro and in vivo both in clinical and experimental conditions.

Projected studies: Migration of cells and gradual types of ovary explant may have interferred with differentiation. It is proposed to plant tissue in rapidly rotating roller drum in chemically defined medium in order to obtain survival and growth but to minimize massive proliferation and migration of the cells.

Study B. Title: Studies in the cultivation of human endocrine secreting tissue in vitro. To cultivate cells of human endocrine tissue as made available by the Endocrinology Branch of the National Cancer Institute in order to learn the extent and duration of hormone secretion of isolated cell types in vitro.

Methods employed: Human biopsy material from the Endocrinology Branch. Among these have been three chorioepithelioma, two pancreatic adenomas, two hydatidiform mole and two adrenal tumors. After the tissue was dissociated into small cell clumps, these were planted in vitro. Human serum was invariably used on some cultures either supplemented with chemically defined protein-free medium NCTC 109 or with embryo extract. Occasionally with some cultures a plasma clot was used but with some, perforated cellophane was employed. Photographic records were kept on cell types. Assays were made on fluids and or cells from each strain planted; transient positive results were obtained from 1 chorioephithelioma and from 1 hydatid mole. At the present time this hydatid is still surviving after 6 months in vitro. Endocrine assays for gonadotropines are in progress. Similarly the choroepithelioma is still surviving in vitro after 2 months. No activity has been demonstrated as evidenced by chemical assay for cortico-steroids on the adrenal tissue. The fluid from one culture of pancreatic tumor of the Islet cells in chemically defined medium with perforated cellophane gave some evidence

of continued activity in vitro. This particular tumor had been diagnosed as an islet cell tumor when the patient after surgery had showed recovery from severe hypoglycema.

Significance: The development of techniques for growing human endocrine tissues in vitro so that they continue to function should have considerable practical value for therapy. From the point of view of cancer research any endocrine assays or data from histochemical assays should serve as valuable indices of the changes in cells in vivo and in vitro both in clinical and experimental conditions.

Projected studies: Experiments will be continued to culture various tissues of endocrine origin as they are made available from services in the Clinical Center and other sources. Methods must be developed for culturing such highly specialized cells. The duration and amount of hormone secretion and study of the morphology of the tissue growth with photographic records will be made. Histochemistry biochemical analyses and morphology and mouse chamber techniques will be employed and correlated with function and therapy. Attempts will be made to devise new methods of assay of function by grafting as suggested by mouse chamber techniques and as justified by the growth of cells in vitro.

Study C. Title: The low temperature survival and subsequent cultivation of human endocrine secreting tissue in vitro.

Objective: To preserve tissues such as ovary testes, thyroid, parathyroid, pituitary, pancreas, adrenal, spleen and liver in a viable state, using the technique of Parkes, Polge and Smith, by impregnating with 15% Glycerine-Saline solution and subsequent freezing to -79°C.

Methods employed: Endocrine tissue is removed, a section fixed for histological study; a strip explant of the tissue is then planted in tissue culture to be used as fresh control and the remainder of each tissue is impregnated with 15% glycerine-Earle's saline for 1 hour and then frozen in dry ice alcohol slush (-79°C) for 1 hour. The tissue is subsequently thawed in a 40°C water bath and then planted in Carrel D-3.5 flasks.

Major findings: 1) In all instances with the exception of the pancreatic tissue, outgrowth was obtained from the glycerine impregnated frozen tissue within 7 days in vitro. The pancreas in these studies failed to exhibit cell outgrowth but did show a luxurious growth of an unidentified fungus-like micro-organism in the 4 cases studied.

Significance: The significance of the uniform findings of a fungus-like contamination is not clear. No data were obtained as to whether any of these tissues studied had maintained their secretory function. Correlation of cell outgrowth with the histological preparations has not yet been completed.

Projected studies: 1) Close the studies already initiated, and correlate the outgrowth of the cell explants with the histological preparations. 2) Attempts to establish more satisfactory methods of cultivating the endocrine tissue with a maximal degree of functional tissue differentiation. This would eventually look forward to possibilities of clinical application through collaboration with the Tissue Bank, National Naval Medical Center.

Study D. Title: A study of long term human skin strains in tissue culture.

Objective: Use of cells of an established strain of human skin epithelium as a therapeutic dressing on denuded skin areas of experimental animals. 2) To evaluate in preliminary fashion any tendency of such implanted human skin epithelial cells to give rise to malignant neoplasms at the unplanted sites.

Methods employed: 1) The development of the human skin strain has been described in earlier annual reports. In the past fiscal year approximately 15 hamsters and 20 rabbits have received cell suspension transplants of the human skin epithelial cells over denuded skin areas. All animals were cortisone treated to minimize the heterograft reponse. 2) A study utilizing 268 golden hamsters was initiated using the technique of Toolan of injecting the cells into hamster cheek pouches. The animals were divided into 4 groups; each animal in these 4 groups was injected bilaterally with one of 4 types of cells respectively; (a) the HeLa strain of human cervial carcinoma, (b) mouse sarcoma cell NCTC Clone L-929, (c) NCTC Strain 1769 and its substrain NCTC Strain 2198, (d) which has been cultivated in entirely heterologous media. Each group of animals was divided further into 3 subgroups as determined in the frequency of cortisone given (a) animals which received 5 mgm cortisone weekly, (b) animals which received 5 mgm. cortisone at the time of cell implantation only, and (c) animals which received no cortisone. Groups of animals were sacrificed at weekly intervals for as long as nine months.

Major findings: 1) There was no fluid loss at the operative site of the animals receiving the suspension of tissue culture skin, providing the site was free of gross infection when the

implanted cell population per operative site (measuring approximately 4 sq. inches) exceeded 40 million cells. Viable cells were found after 21 days in situ; serious exudation over the area was slight. In the control animals, e.g., where an operative defect was created, but no cells were added to the defect, the observation chambers became partially filled with serous exudate. 2) Histological preparations of the growths produced in vivo were submitted to several pathologists for study. It was the concensus that the growth produced by the human skin epithelial cells did not appear malignant.

Significance: 1) These extremely preliminary results suggest that the human skin cells when used as a suspension on a defined operative defect may have helped prevent the serious fluid loss usually associated with the open wounds in the controls. 2) There were no indications from this as yet incomplete study that the human skin epithelial cells had undergone a malignant transformation after more than 2 years in culture.

Projected studies: 1) Complete study of tissues now being processed. 2) Continue collaboration with the Tissue Bank of the National Naval Medical Center in study of the potential use of this strain of epithelial cells in covering denuded skin areas.

Study E. Title: Viability of tissue cultured cells of human skin epithelium exposed to varying freezing velocities and depths of freezing.

Objective: To determine the viability of cells in vitro under different conditions of preservation.

Methods employed: Roller tube cultures of an established strain of human skin epithelial cells were subjected to (1) ultra rapid freezing in liquid nitrogen (-195°C) for 45 minutes, (2) rapid freezing in a mixture of dry ice and 95% ethyl alcohol (-79°C) for 1 hour, (3) to slow freezing in a dry ice chest (-79°C) for 1 hour, (4) slow freezing in a commercial food freezer (-20°C) for 1 hour. All cultures were thawed at 40°C in a water bath, but no cells were exposed to a temperature above 37°C. Control cultures were stored for 1 hour at room temperature followed by water bath treatment. Human serum nutrient medium was then returned to cultures. After 120 hours, fresh daughter cultures were prepared and 24 hours later an evaluation of conditions of cultures was made. For glycerine protection study tissue was impregnated with 15% glycerine-Earle's saline for 1 hour and frozen at (-79°C) for 1 hour. Strip explants were then planted in plasma clot substrate cultures in dilute human serum-chick embryo extract.

Major findings: All cultures under all conditions described above were found to be viable. Viable cells in cultures subjected to commercial freezer temperatures were limited in number. Glycerine impregnation of fresh tissue showed viability of tissue as evidenced by cell outgrowth or migration from the explants with one exception.

Significance: The preservation of living tissue cells, both from established cell strains and comparatively fresh tissue, has been under study by the Tissue Bank, National Naval Medical Center as of possible value in reparative surgery.

Projected studies: Use for reparative surgery may be tested at a future date.

Study F. Title: The utilization of a long term strain of human skin epithelium for the propagation of certain arthropod borne viruses.

Objective: Propagation of the arthropod borne viruses in this particular strain as demonstrated by the Japanese B. Encephalitis, Western Equine Encephalomyelitis, West Nile Virus and Dengue type 1 virus. To demonstrate possible cytopathogenic effect by the viral agents which might be utilized for diagnostic purposes, and the propagation of agents in high titer relatively free of extraneous materials for use in immunological, biophysical and related studies.

Methods employed: At the NMRI the agents were inoculated in human skin epithelium at known titer and permitted to incubate for 30 minutes, after which time the tissue was washed 5 or 6 times to free the fluid of any unabsorbed viruses. The viral inoculated tissue cultures were incubated at either 32 or 36° for varying intervals of time, and the supernatant was harvested for titer of viruses by intercerebral inoculation of white Swiss mice, and the cultures were periodically observed for any cytopathogenic effect. Various experiments were undertaken, whereby the tissue cultures were grown on coverslips and studied histologically after being stained as a plaque technique.

Major findings: That Western Equine encephalomyelitis and Japanese B Encephalitis propagate well in the human skin cultures with the production of cytopathogenic effects. West Nile and Dengue have not been titered as yet.

Significance: This is a very promising tool and if the cytopathogenic effect observed is specific, this could prove to have diagnostic significance. The titers as so determined is exceptionally good, and the production of purified viruses for vaccine is promising.

Projected studies: Limited continuation of the present studies by personnel of the N. N. M. C., and establishment of a tissue culture propagated strain of viruses by continuous passage of the Japanese B encephalitis, in particular.

Part B: Budget Data: 15. 430

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Long-term tissue culture of human skin. Vernon P. Perry, Virginia J. Evans, Wilton R. Earle, George W. Hyatt and Wallace C. Bedell. Am. J. Hygiene, 63: 52-58, 1956.

Storage. Vernon P. Perry, Virginia J. Evans, George W. Hyatt, Wilton R. Earle, and Jerry W. Draheim. Transplantation Bulletin, 3: 51-52, 1956.

The viability of fresh and frozen cornea as determined in tissue culture. Samuel D. McPherson, Jerry W. Draheim, Virginia J. Evans, Vernon P. Perry, and Wilton R. Earle. Am. J. Ophthalmology, 41: 513-520, 1956.

Characteristics and potentials of long-term cultures of human skin. Bassett, C.A., Evans, V.J., Earle, W.R. Plastic and Reconstructive Surgery, 17: 421-429, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 1130
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$20,090	$6,630	$26,720	1/2	2	2 1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	2	2 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Tissue Bank, National Naval Medical Center, Naval Medical Research Institute, National Naval Medical Center.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 431
SERIAL NUMBER

2. National Cancer
INSTITUTE OR DIVISION

3. Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Tissue Culture Section
SECTION OR SERVICE

5. Bethesda, Maryland
LOCATION (IF OTHER THAN BETHESDA)

6. Study on cell transformations in vitro.
PROJECT TITLE

7. Study A - Sanford, K. K. and Andervont, H. B.
Study B - Sanford, K. K.
Study C - Evans, V. J.
PRINCIPAL INVESTIGATOR

8. Hobbs, G. L., Hawkins, N. M., and Earle, W.R.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS): Study A. Title: The relation of the milk factor to the malignant cell in culture.

Objective: To determine whether the milk factor persists in tissue cultures of spontaneous mammary carcinoma of the strain C3H mouse.

Methods employed: Cells were cultured from several spontaneous mammary carcinomas of the strain C3H mouse. Assays of the cells for the milk factor were made by Dr. Andervont at intervals during cell growth in vitro. Single cells were isolated and grown into cultures by cloning procedures. Tissue culture cells were injected into x-irradiated strain C3H mice.

(Use reverse and additional pages, if necessary)

Major findings: The tissue culture cells appeared to lose the milk agent after several transfer generations in vitro. The cultures gave rise to typical "sarcomas" when injected into x-irradiated hosts. When three clones were established, however, from the mixed cultures, all three gave rise to carcinomas.

Significance: The role of viruses or virus-like particles in carcinogenesis has not been clear. The question has arisen as to whether the milk factor acts merely as a carcinogen in initiating the malignant transformation or whether the agent must proliferate in the cell for the continued malignancy of the cells. The results of this study throw some light on the problem.

Projected studies: To continue and complete the studies outlined above.

Study B. Title: Characteristics of lines of cells derived in vitro from a single adult mouse cell.

Objectives: 1) To determine the physiologic differences between lines of cells that arose from the same single cell but differ in their capacity to produce sarcomas when injected into mice. 2) To establish and analyze the change in sarcoma-producing capacity induced in these cells by mouse passage.

Methods employed: Quantitative procedures in which known numbers of cells were injected into x-irradiated and non-irradiated, immunized and non-immunized mice have been used for biological assay of the sarcoma-producing capacity of the cells. Proliferation rates in vitro were studied by the use of quantitative replicate culture procedures. Isolation of cell types was made by cloning procedures. In collaboration with Dr. Mark Woods the energy metabolism of the cell was studied. In collaboration with Dr. Ruth Merwin the growth in vivo in transparent chambers was followed. Dr. A. J. Dalton is studying the mitochondria of the cells by electron microscopy.

Major findings: 1) Cells of both lines were found to immunize mice of the inbred strain from which the single cell originated. 2) Although cells of the two lines proliferated at comparable rates in vitro in a culture fluid of dilute horse serum and chick embryo extract, the growth of cells of the two lines in vivo was significantly different. 3) The difference in growth in vivo appeared as early as 24 to 72 hours, after cell injection in x-irradiated mice before the development of immunity could be demonstrated. 4) The metabolism of cells of the two lines differed. 5) The vascular response of the host to cells of the two lines differed. 6) The numbers of mitochondria in

cells of the two lines appear to differ. 7) After one generation of growth in vivo cells of the low sarcoma-producing line changed to resemble those of the high sarcoma-producing line. 8) From the two lines of cell clones have been established that are being studied to determine whether the population of cells in each line is homogeneous or whether variants are present that could be selectively propagated in the mouse.

Significance: This analysis of the behavior of cell lines that differ in their ability to produce sarcomas in the strain C3H mouse and that have transformed from normal to tumor-producing cells should provide some information as to the type of changes that these cells have undergone in their transformation.

Projected studies: 1) To complete the studies above. 2) To determine whether the nutritional requirements of the two cell lines differ in an effort to explain the contrasting growth rates in vivo. 3) To start with a clone of normal tissue cells in chemically defined medium and follow the biochemical, metabolic, and carcinogenic transformations of the cells.

Cooperation with Dr. Mark Woods and Dr. Dean Burk, Laboratory of Biochemistry with Dr. Ruth Merwin and Dr. A. J. Dalton, Laboratory of Biology, N.C.I.

Study C. Title: Study of the strain of mouse liver cells from the same single cell.

Objective: To study two substrains arising from a common clone of mouse liver epithelium. One of these strains (1795) is of high tumor producing ability on reinoculation into mice of the same strain; the other is low in tumor production and is designated clone 1469. The primary objective is to make available two strains of epithelial cells of common clonal origin for comparing their metabolic and physiological characteristics.

Methods employed: Cell cultures have been maintained in vitro for study. Cultures injected into both x-irradiated and non-irradiated C3H mice have been observed over prolonged periods exceeding a year. Photographic records have been kept.

Major finding: Mouse liver NCTC Clone 1469 was originated from NCTC Strain 721 in April 1948, and this clone strain has shown a low capacity for developing tumors on injection; only two tumors have been found in x-irradiated mice in a comparatively large series of mice. Practically 100% tumor incidence was obtained from one of these tumors on trocar injection into both non-irradiated and x-irradiated mice. Tissue from one of

these tumors arising from injection of NCTC Clone 1469 cells was grown to produce NCTC Strain 1795. This strain of cells on reinjection into C_3H mice gave a comparatively high incidence of tumors (exceeding 50%) both in x-irradiated and non-irradiated mice. The use of x-irradiated mice did not significantly increase the number of tumors observed. Tumor tissue on subinoculation gave 100% tumors in irradiated mice while those in non-irradiated mice was only 10%. Detailed comprehensive reports on the comparative metabolism of these three strains of cells 721, 1469 and 1795 will be reported by Dr. Westfall with whom collaborative studies have been carried out in order to characterize the metabolism of these cell strains. Similarly, sections from all tumors have been diagnosed by the Pathology Laboratory as highly differentiated epithelial cells not unlike anaplastic hepatomas. Most recently the incidence of tumors obtained from injection of 1795 cells has dropped to 0.

Significance: Comparative studies of the metabolism and physiology of these three cell strains 721, 1469 and 1795 appear to be of significance in understanding the characteristics of transition from a strain of cells giving no tumors under the conditions of injection to one giving less than 1% incidence to a third strain giving a high incidence which ultimately loses its ability to produce any tumors.

Projected studies: Metabolism studies will be continued. Studies on injections will be continued to determine if tumors can be obtained by use of larger populations on injection. If new tumors are obtained a new strain must be set and recloned immediately and metabolism studies must parallel injection studies.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 431
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$28,110	$9,280	$37,390	1½	2	3 ½

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1 1/2	2	3 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None.

(Use reverse and additional pages, if necessary)

Part C: HONORS, AWARDS & PUBLICATIONS 15. 431

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

The tumor-producing capacity of strain L mouse cells after ten years in vitro. Katherine K. Sanford, Gwendolyn L. Hobbs, and Wilton R. Earle. Cancer Research, 16: 162-166, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 432
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Tissue Culture Section
SECTION OR SERVICE

5. Bethesda, Maryland
LOCATION (IF OTHER THAN BETHESDA)

6. Comparison Microcinematograph
PROJECT TITLE

7. Earle, W. R.
PRINCIPAL INVESTIGATOR

8. McQuilkin, W. T.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS): Construction and operation of a 3 optical system comparison microcinematograph specially designed for quantitative comparative studies of normal and malignant cells on tissue culture.

Progress Report: Test film strips and preliminary films have been taken on the microcinematograph. These tests have indicated the necessity for extensive changes in the control and operational mechanisms. In collaboration with the Instrument Design Section, NIH, changes are now under way or under design which it is hoped and expected should eliminate these inadequacies of function and have the machine in active effective operation early in 1957.

(Use reverse and additional pages, if necessary)

The construction and testing of this instrument has now extended over a number of years and has been beset by many harassing difficulties. Some of these have been due to long delays resulting from lack of trained personnel for testing and operation of the instrument, and to delays resulting from necessity of training personnel in this specialized field. The present difficulties, however, are those of design and accuracy and reliability of operation, and arise from the fact that with such a continuous operation precision instrument practically every feature of the drive mechanism, the automatic illumination control devices, and the integration of the control of the various optical system mechanisms had to be worked out, modified, and tested for this particular field of research. The work has been further handicapped by the changing systems of instrumentation organization which have occurred in the NCI and NIH during the construction and testing of this machine. In spite of the handicap encountered I have every confidence that this instrumentation is well justified and that it should play an extremely significant role in clarifying the nature of the many complex cell transformations and alterations of cell behavior and function which occur in living tissue cells in vitro. It is in this belief that I am pressing for completion of necessary changes as expeditiously as possible.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 432
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$8,040	$2,640	$10,680	1/3	2 3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Instrument Design Section, NIH

(Use reverse and additional pages, if necessary)

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 433
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Study on viral agents recovered from mouse neoplasms with reference to their possible role in the following: (a) etiology of mouse leukemia and related tumors, and (b) as they relate to tumors and chemotherapy studies.
PROJECT TITLE

7. Sarah E. Stewart
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Monkey kidney tissue culture, which is used routinely by virologists for the propagation of human and animal viruses was used as a system for attempting to demonstrate viruses in mouse tumors and to determine the relationship of such viruses to tumor induction. The virus of lymphocytic choriomeningitis was recovered from some of the preparations that induced tumors, but no relationship between this virus and tumor induction was conclusively demonstrated. However, by the tissue culture method, we were able to demonstrate a subcellular agent derived from mouse leukemias and parotid gland tumors which induced neoplasms in mice inoculated with it. The agent has not been identified. The neoplasms obtained by this method are of unusual types and are as

(Use reverse and additional pages, if necessary)

follows: tumors of the parotid, submaxillary, and sublingual glands, epithelioid thymic tumors, adrenal medullary tumors, and early mammary tumors. All mice bearing parotid gland tumors also had multiple proliferative renal cortical lesions of the convoluted tubules. Control mice receiving inocula of fluids from monkey kidney tissue cultures not inoculated with tumor material have remained free of neoplasms.

Lymphocytic choriomeningitis virus was recovered from 2 sublines of L1210 leukemias. One subline, AMD,Fx which was reported to be dependent on a-methopterin for optimal growth, grew well in the absence of the drug if the hosts were immunized to LCM virus before receiving the tumor. LCM virus was found to have a destructive effect on the tumor; this effect was prevented by the drug. It was also found that a-methopterin would prevent the pathogenic effect of the LCM virus in mice inoculated intracerebrally with many lethal doses of the virus.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 433
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$24,100	$7,940	$32,040	1	2	3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	2	3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. 433
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Stewart, S. E. and Haas, V. H.: Lymphocytic choriomeningitis virus in mouse neoplasms. J. Nat. Cancer Inst. 17: 233-245, 1956.

(2) Haas, V. H. and Stewart, S. E.: Sparing effect of a-methopterin and guanazolo in mice infected with virus of lymphocytic choriomeningitis. Virology. 2: 511-516, 1956.

(3) Haas, V. H., Stewart, S. E., and Briggs, G. M.: Folic acid deficiency and the sparing of mice infected with the virus of lymphocytic choriomeningitis. Virology. In press.

(4) Stewart, S. E., Eddy, B. E., Gochenour, A. M., Borgese, N. G., and Grubbs, G. E.: The induction of neoplasms with a substance released from mouse tumors by tissue culture. Virology. In press.

17. LIST OF HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 434
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Biology
LABORATORY, BRANCH, OR DEPARTMENT

4. Tissue Culture Section
SECTION OR SERVICE

5. Bethesda, Maryland
LOCATION (IF OTHER THAN BETHESDA)

6. Metabolic studies on tissue cells in vitro.
PROJECT TITLE

7. Study A - Westfall, B.B.
Study B - Westfall, B.B.
Study C - Earle, W.R. and Bryant, J.C.
Study D - Burk, D., Evans, V.J., Woods, M.W. and Earle, W.R.
PRINCIPAL INVESTIGATOR

8. Wight, K M., Peppers, E.V., Evans, V.J., Sanford, K.K., Schilling, E.L. Hobbs, G.L., Hawkins, N.M., McQuilkin, W.T., Kerr, H. and Earle, W.R.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS): Study A. Title: Study of metabolism of tissue cells in large agitated fluid suspension cultures, with special respect to glycogen metabolism.

Objective: To ascertain to what extent certain cell strains after long cultivation in vitro retain characteristic functional patterns, in this instance that of glycogen deposition. Also to make a study of the materials used for glycogen formation by such cells after long cultivation and during periods of adaptation to growth in vitro.

Methods employed: Certain cell strains, e.g. liver (mouse) and human epidermis are examined for their glycogen content by the Pfluger total glycogen concentration as found following digestion of the tissue with

(Use reverse and additional pages, if necessary)

strong KOH. Comparison is also made between the strains using TCA extraction of the tissue for getting out the carbohydrate. Cells are examined at different times following fluid change and in different media.

Major findings: A paper is now being prepared on the glycogen content of 8 cell strains at 48 hours after fluid change. It was found that the three strains derived from liver, 721, 1469 and 1795 had relatively high glycogen content, HeLa likewise and the human epidermis under these circumstances also.

Projected studies: It is planned to extend the study to include such precursors of glycogen as formate (tagged) and pyruvate (tagged) etc. so as to investigate mechanisms and utilizable substrates.

Study B. Title: Study of metabolism of tissue cells in large agitated fluid suspension cultures, with special respect to enzyme patterns.

Objectives: 1) To ascertain to what extent strains of cells grown for long intervals in tissue culture retain the enzymic pattern and activity of the cells of origin. 2) To use enzyme patterns as an aid in the identification of cells after long growth in vitro.

Methods employed: Cells are grown in sufficient quantity e.g. 100 to 1000 mg. in culture and enzymic patterns examined by modification of standard procedures: e.g. arginase estimated by estimation of urea formed using the procedure of Archibald for urea with excess arginine added to tissue extract.

Major findings: The strains of liver cells existing in the laboratory have been found to possess arginase activity comparable to that of the liver of the mouse of origin. HeLa cells show much less activity than liver and L none at all. All five strains have been found to have considerable rhodanese activity.

Projected studies: The present program includes plans for extending the study of enzyme patterns to other cell strains and to examine other enzyme activities.

Study C. Title: Glucose utilization and cell proliferation of cell suspensions in tissue culture.

Objective: To establish correlations between culture population density increase and glucose utilization under controlled experimental conditions in rapidly agitated fluid suspension

of various strains of animal tissue cells.

Methods employed: The large agitated cultures of cell suspensions of several different cell strains were sampled at fluid change or fluid addition every two or three days. The 1 ml. sample for reducing sugar determination was taken directly from the cell suspension. The glucose in the fresh medium, a known value, compared with the reducing sugar found in the old fluid two or three days later, gives a measure of glucose utilization during that time. Changes in population density were determined by nuclear enumerations of aliquots of the culture.

The logarithmic phase of the growth curves is evaluated in terms of generation time by means of formulae for exponential growth curves.

Major findings: Cultures of certain cell strains such as human skin consistently use more glucose than do mouse fibroblast strain L, whether expressed as average glucose used per million cells increase, or average glucose used per million cell days. Various levels of glucose are used in the fresh media, ranging from the standard concentration of 100 milligram percent in Earle's saline and in serum up to 600 mg. percent, with 260 mg. percent as the level most frequently used. In all cases 260 mg. percent glucose in fresh media prevents exhaustion of the glucose by the cells before the next fluid change, while a level of only 100 mg. percent glucose frequently results in complete removal of the glucose and, frequently, inhibition of cell proliferation.

Average time for doubling the number of cells in the culture ranged from 24 hours to as long as six or seven days, with the majority of the 40 cultures studied having a generation time of about 48 hours during the logarithmic phase. Correlation between glucose utilization and generation time was not significant in an overall statistical analysis of variance of the data for 40 cultures, 6 cell strains, and several kinds of media. Within one cell strain and one kind of media the correlation was good, as a rule. Chemically defined NCTC 109 with 20% horse serum gave a shorter generation time, ca. 24 hours, than other strain or medium. Repeated cycles of rapid logarithmic growth and harvesting 60 percent were carried out with Strain L.

Projected studies: This investigation established a definite correlation between cell population increase and glucose utilization. This basic relationship is being extended to a consideration of the pH trend in the cultures. The constant maintenance of a series of cultures in the logarithmic phase of cell proliferation for rapid subculturing and harvesting

will be placed on a more dependable basis by these developments.

Study D. Title: Mamometric studies on mouse and human cells in vitro.

Objective: To study by mamometric methods the anaerobic and aerobic glycogen, etc.

Methods employed: Over 250 T-60 flask cultures of NCTC Clone 929 of Earle's strain L, human skin epithelium, three lines of mouse liver epithelium, 2 lines of HeLa cells derived from adeno carcinoma of the cervix, 2 lines of human adrenal tumor, Islet cell tumor of the pancreas of the human were maintained in vitro and submitted to the collaborating chemists for their analyses. Nuclei in aliquots of the cell suspensions used by them were counted for correlation with N determination of similar aliquots made by the chemists.

Major findings: The metabolic analyses to date indicate that cell strains of tissue cultures tested show a metabolism previously found to be characteristic of cancer cells (according to the concepts of Warburg and Burk) ranging from strain L and HeLa at the lower order of malignancy for the human strains to the cell of mouse clone NCTC-1742 at the highest order of malignancy where the metabolism of ascites cancer cell is approximated.

Significance: Other unknown things not intervening, according to Dr. Burk, these cells in vitro have metabolism which would support malignancy; but it must be recognized that tissue culture cells in tissue culture medium may have different norms of mtabolic malignancy than previously observed in solid cancers. The study is being continued.

Projected studies: See report of Dr. Burk and Dr. Wight, Cytochemistry Section, Laboratory of Biochemistry.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 434
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$24,100	$7,940	$32,040			

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1 1/3	1 2/3	3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Tissue Bank, National Naval Medical Center.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Effect of glutamine on the growth and metabolism of liver cells in vitro. Benton B. Westfall, Virginia J. Evans, Ellison V. Peppers, Naomi M. Hawkins and Wilton R. Earle. J. Nat. Cancer Inst., 17: 131-138, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

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October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 500
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Pathology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Histogenesis and Pathology of Induced and Spontaneous Tumors of Laboratory Animals
PROJECT TITLE

7. Dr. Harold L. Stewart
PRINCIPAL INVESTIGATOR

8. Dr. Katharine C. Snell
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To gain knowledge of the etiologic and pathogenic factors involved in the production of neoplasms in experimental animals and to make this information available to other investigators.

Methods Employed: 1. A fascicle on transplantable and transmissible tumors of animals has been prepared for publication in the Atlas of Tumor Pathology under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council. This fascicle includes the history, behavior in transplantation, detailed pathologic description, and photomicrographs of 50 of the most widely used animal tumors. (Dr. Lucia Dunham has collaborated on this work.)

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

2. A manuscript entitled "The Pathology of Experimental Cancer of the Liver in Rats: A Critical Review" is being completed for publication in the revised edition of The Physiopathology of Cancer (F. Homburger and W. H. Fishman, editors), and also in ACTA - Unio Internationalis Contra Cancrum. Preparation of this manuscript has involved the examination of histologic sections of the livers of rats fed various carcinogenic azo dyes, the taking of photomicrographs, and a comprehensive review of the literature.

3. A series of untreated rats of 7 strains, males and females, breeders and non-breeders, is being necropsied at various age intervals and subjected to detailed pathologic examination in order to enumerate and classify spontaneous tumors, to study tissue changes with increasing age, and to furnish controls for present and future experiments.

4. A study is being made of the lungs of DBA mice fed 1,2,5,6-dibenzanthracene to ascertain whether this substance induces adenomatosis.

5. Monkeys that received injections of carcinogenic substances into the wall of the stomach several years previously are being necropsied and studied to ascertain whether these substances may have produced gastric neoplasms.

6. A study is being made of the tumors occurring in the NHO strain of mice. These mice are of interest because their ancestors received a single dose of methylcholanthrene in an effort to produce an hereditary gastric carcinoma.

7. The effect of subcutaneous injections of cortisone acetate on the blood vessels and other organs and tissues of hamsters is being studied, and in conjunction with this, observations on the histology of the tissues and organs of the untreated hamster are being made.

Major Findings: 1. Fascicle on Transplantable and Transmissible Tumors of Animals: The completed manuscript together with 300 illustrations was submitted to the editors of the Atlas of Tumor Pathology in February, 1956. It has since been reviewed by special critics and by the members of the Subcommittee on Oncology; it has been corrected by the authors, and is now being prepared for printing. Date of publication is uncertain.

2. The Pathology of Experimental Cancer of the Liver in Rats: A paper based on this study was presented by Dr. Stewart at the Symposium on Liver Cancer at Kampala, Uganda, in August 1956, under the auspices of Unio Internationalis Contra Cancrum. For this presentation approximately 100 colored lantern slides were prepared. An expansion of the original manuscript is being completed, photomicrographs in black and white have been taken, and within the next month the paper will be submitted to the editors of the Physiopathology of Cancer. The paper will also be published in Acta - Unio Internationalis Contra Cancrum.

10. PROJECT DESCRIPTION (Cont'd)

3. A total of 429 untreated rats have been necropsied. The series of necropsies is complete on the Marshall 520, the A x C, the Osborne-Mendel, and the Buffalo strains. No histologic slides have been examined since 1954.

4. Necropsies have been completed on the DBA mice fed 1,2,5,6-dibenzanthracene. No histologis sections have been examined, and therefore no significant findings can be reported.

5. No monkeys necropsied to date have shown gastric carcinoma nor any lesions that can be definitely attributed to the injection of carcinogenic substances into the wall of the stomach. However, one monkey in August 1956 developed the blood picture and physical findings of pernicious-like anemia. X-rays showed no gastric lesion, but it may be significant that this monkey has developed symptoms that are thought sometimes to precede the development of carcinoma of the stomach in human beings. The monkey is being treated for pernicious-like anemia and is being observed closely.

6. Necropsies on NHO mice have been completed. No carcinomas of the glandular stomach have been found, but various other tumors that may be of interest are being carried in transplantation.

7. It has been found that approximately 70 percent of hamsters receiving cortisone develop dissecting aneurysms of the aorta; the possible mechanism for this is being studied. No hamsters have shown glycosuria (as is the case with cortisone-treated rabbits), but various changes in the pancreas, kidneys, adrenal glands, and gastrointestinal tract have been observed and will be studied in detail in the future.

Significance to Cancer Research: The knowledge gained from a study of neoplasms in laboratory animals should provide a basis for further work on the mechanism of carcinogenesis in human beings.

Proposed Course of Project: Publication of the study of the pathology of experimental cancer of the liver in rats should be made in 1957. Examination of histologic sections of experiments enumerated as parts 3-7 will be continued or initiated.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 500
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$43,320	$17,260	$60,580	2	3-1/2	5-1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2	3 1/2	5 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 500
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Stewart, H. L., Snell, K. C., Dunham, L. J., and Schlyen, S. M.
Transplantable and Transmissible Tumors of Animals
Fascicle 40 of the Atlas of Tumor Pathology.
A.F.I.P., Washington, D. C. (In press)

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 501 (a)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Pathology Section
SECTION OR SERVICE

5. New York, Israel, Washington, D.C.
LOCATION (IF OTHER THAN BETHESDA)

6. Geographic pathology field studies on uterine cancer in New York City, Israel, and Washington, D.C.
PROJECT TITLE

7. Dr. Lucia J. Dunham, Dr. Harold F. Dorn, Dr. Harold L. Stewart
PRINCIPAL INVESTIGATOR

8. Dr. John H. Edgcomb, Dr. Louis B. Thomas
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Field studies of uterine cancer (cervix and corpus) in New York City, Israel, and Washington, D.C.

Objectives: The objective of this project is to complete an extensive questionnaire study of women with uterine cancers in different geographic areas and racial groups, and to analyze the data obtained with a view to identifying factors suspected of predisposing to or causing female genital cancers. Also, basic data on the incidence and pathologic (microscopic) diagnoses of the cancers are obtained.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Methods: For the area under study and the period of study all patients possible with the diagnosis of uterine cancer are interviewed, and an equal number of control patients as well. The confidential data recorded include social, medical, and surgical history, and menstrual, marital, and pregnancy data. Records are kept of the small number of patients not interviewed, their diagnoses, and the reasons for failure to interview. Pathologic material is studied in all cases.

A coding statistician and two medical social worker interviewers are currently employed in the project. In addition, assistance is obtained from a statistician, statistical clerk, and from the Statistical Processing Section.

Major findings: 1. Data from 1872 interviews with Jewish women in Israel have been put on IBM cards and tabulated.

2. Data from 1642 interviews with Jewish and non-Jewish white women in New York City have been put on IBM punch cards and tabulated.

3. Data from 714 interviews of non-white women in New York City have been reviewed and coded.

4. Data from about 214 interviews of non-white women in Washington, D.C., have been reviewed and coded.

5. Extensive tabulations and worktables have been prepared in the studies listed under #1 and #2. Analysis of the material and preparation of reports for publication are in progress.

6. Pathologic material has so far been examined in about 90 percent of the above cases.

7. The medical literature on geographic and environmental factors in uterine cancer has been reviewed.

Significance to cancer research: The significance of the study lies in the hope of supplying a sound scientific basis for the understanding of factors of custom,habit, or environment as they may be related to relatively high frequencies of uterine cancers in some groups of women as contrasted to intermediate or relatively low frequencies in other groups.

Proposed course of the project: The proposed course is to tabulate, analyze, and appraise the material referred to above, and to prepare reports of these studies for publication. At the conclusion of the project it is planned to undertake clinical studies in geographic pathology on patients with cancers of the esophagus, stomach, and large bowel.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 501 (a)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,850	$230	$12,080	1-1/2	0	1-1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1 1/2	0	1 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Office of Biometry, Office of the Director, National Institutes of Health and Biometry Section, Biometry and Epidemiology Branch, National Cancer Institute.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 501 (a)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Stewart, H. L., Snell, K. C., Dunham, L. J., and Schlyen, S. M. Fascicle for Atlas of Tumor Pathology: "Transplantable and Transmissible Tumors of Animals". In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

None

Form No. ORP-1
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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 501 (b)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Pathology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Geographic Pathology. Experimental studies relating to the geographic pathology of mouth cancer.
PROJECT TITLE

7. Dr. Lucia J. Dunham
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Experimental studies on mouth cancer.

Objectives: The objective of this project is to examine the mucous membrane of the hamster cheek pouch for proliferative changes that may develop when test substances are chronically maintained in contact with these surfaces. Substances used are environmental materials suspected of being mildly carcinogenic for man.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Methods: The pellets which have been prepared for insertion in the cheek pouch of the hamster include the tobacco, lime, areca nut, and pepper leaf of the Far Eastern "betel quid", the plastic material used in preparing "false" teeth, and tobacco tars and control materials. A technical assistant is retained part-time for help in the study.

Major Findings: Since the project is a long-term one, there are no major findings at present. It has been demonstrated that proliferative changes can be initiated on this mucus surface. It is hoped to maintain the experimental animals for at least two years before gross and microscopic study of the pouches. The operations used for testing substances have so far been performed on more than 300 hamsters.

Significance to Cancer Research: The significance is to reach an improved understanding of chronic irritant factors as they may relate to cancer of the mouth in man. In addition it will be ascertained whether or not the technique is useful for testing suspected weak carcinogens in general.

Proposed Course of Project: The study will be extended in any direction indicated by the findings. Both the cheek pouch under study, and the opposite normal pouch will be studied with care when the experimental hamster becomes ill or dies. In addition, improved techniques of study will be attempted.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 501 (b)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$23,690	$9,440	$33,130	1-1/2	1-1/2	3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1 1/2	1 1/2	3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 16 or 17

(Use reverse and additional pages, if necessary)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 502
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Histogenesis and histopathology of cutaneous neoplasms.
PROJECT TITLE

7. Dr. John H. Edgcomb
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

During the past year the project has included the following activities:

A. Studies of diseases of man (collaborative research).

i. Mycosis fungoides

The reaction to electron beam therapy of the skin of seven patients with mycosis fungoides has been studied pathologically. The therapy clears the epidermis and superficial dermis of mycosis infiltrates. A report of these observations is being submitted to the International Congress of Dermatology, which meets in Stockholm in August, 1957.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

ii. Melanomas

Ninety-five naevi from the skins of patients with malignant melanomas and twenty naevi from other patients have been examined. There is no indication that naevi from the patients with melanoma are different from those of the control group. The study continues.

B. Studies of diseases in animals.

i. Further studies are being made on C strain mice bearing Dr. R. Bryan's transplantable squamous carcinoma. Many of these mice develop severe leukocytosis. Attempts to transform this disease into granulocytic leukemia have been unsuccessful.

ii. Two melanomas have been produced in guinea pigs by skin painting with dimethylbenzanthracene.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 502
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,850	$4,720	$16,570	0	1½	1½

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	1 1/2	1 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 502
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Andervont, H. B., and Edgcomb, J. H.
Responses of seven inbred strains of mice to percutaneous applications of 3-methylcholanthrene. J. Nat. Cancer Inst. 17: 481-495, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

None

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 503
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Collaborative Research Requiring Pathologic Anatomy.
PROJECT TITLE

7. Dr. Thelma B. Dunn
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To collaborate with and advise other investigators at the National Cancer Institute in phases of research problems requiring experience in animal pathology.

Methods Employed: This may consist in (1) Full responsibility and separate publication as senior author of material reviewed in connection with work of another investigator, (2) Review and description of animals coming to autopsy, and publication of the findings as a separate section of a paper describing the entire experiment, (3) Review and diagnosis of tumors found by investigator in course of experiments, the information to be used in a tabulation of results, (4) Occasional consultation and advice on pathologic aspects or material in an experiment.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 503
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$10,550	$4,200	$14,750	1/3	1	1-1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1	1 1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 503
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Dunn, T. B., Morris, H. P., and Wagner, B. P. Lipemia and glomerular lesions in rats fed diets containing N-N^1-diacetyl- and 4,4-4^1, 4^1-tetramethylbenzidine. Proc. Soc. Exp. Biol. & Med. 91: 105-107, 1956.

Heston, W. E., Deringer, M. K., and Dunn, T. B. Further studies on the relationship between the genotype and the mammary tumor agent in mice. J. Nat. Cancer Inst. 16: 1309-1334, 1956.

Dunn, T. B., Heston, W. E., and Deringer, M. K. Subcutaneous fibrosarcomas in strains C3H and C57BL female mice, and F_1 and backcross hybrids of these strains. J. Nat. Cancer Inst. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

10. PROJECT DESCRIPTION (Cont'd)

Major Findings: (1) A publication describing fibrosarcomas developing in strain C3H mice in an experiment of Dr. Heston's. Only one previous description was found of spontaneous fibrosarcomas in mice, though induced tumors are frequent. (2) Continued work with Dr. Law on animals given cell free substances when newborn. A number of parotid gland tumors have now been found in untreated mice. Collaborative work with Dr. Potter on DBA mice given a variety of treatments has given us an opportunity to study a number of transplantable reticular neoplasms in mice. These show a remarkable individuality both in morphology and biological behavior. The ascites form of these tumors is also being studied. (3) Diagnosis of mammary tumors for Dr. Andervont is continuing. Diagnosis of reticular neoplasms for Dr. Law is continuing. Observations are confirming previously published material. (4) Occasional study of tumors developed from tissue culture in Dr. Earle's laboratory is continuing. We believe that epithelial tumors (hepatomas and mammary tumors) have been successfully carried in culture without transformation to fibrosarcomas.

Significance to Cancer Research: Pathologic review is indispensable to many types of cancer research. This is best carried out by a specialist in pathologic anatomy, since with the present complexities of research, a single investigator cannot know this field and his own specialty.

Proposed Course of Project: This work will continue as in the past. When, as a result of many observations, enough information is accumulated regarding any subject, publications will be made.

Note: In addition to investigators within the Institute, a number of slides have been examined (often with Dr. H. L. Stewart) for investigators outside. Examples are tumors for Dr. Wm. Poel, University of Pittsburgh, a skin lesion for Dr. Eichwald, University of Utah, and slides for Dr. Charlotte Friend, Sloan Kettering Institute.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 504
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induction & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. An Electron Microscopic Investigation of Normal and Neoplastic Tissues.
PROJECT TITLE

7. Dr. William G. Banfield
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To describe the ultra structure of selected neoplasms and to compare it with suitable normal and control tissues.

Methods Employed: The usual electron microscopic methods will be used.

Major Findings: The electron microscope has just been put into operation.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 504
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,950	$1,560	$5,510	1/2	0	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	0	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 504
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Banfield, W. G. Width and length of collagen fibrils during the development of human skin, in granulation tissue and in the skin of adult animals. J. of Gerontol. 10: 13-17, 1955.

Banfield, W. G. Age changes in the swelling capacity of the human Achilles tendon. J. of Gerontol. 11: 372, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 506
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Study of Transplanted Tumors in Mice.
PROJECT TITLE

7. Dr. Thelma B. Dunn
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives. To study various unusual tumors which may throw light on the normal cell counterpart or cell function, and assist in understanding of neoplasia.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Methods Employed: At the present time three tumors are receiving intensive study, (1) A mast cell tumor (with Dr. Michael Potter). This is the first transplantable mast cell tumor ever studied closely. A report is now ready to submit to the Editorial Board. (2) Two transplantable plasma cell neoplasms. These tumors originate in the ileocecal area in strain C3H mice. (3) An adrenal tumor having a lactogenic effect. Study of this tumor has just begun, but it is hoped that the nature of the mammary gland stimulation can be determined.

Major Findings: (1) The mast cell neoplasm has no appreciable effect on the host, even when the tumor attains a large size. Pigmented bodies and hematoidin crystals in the tumor tissue suggest a function in bile pigment formation. (2) One plasma cell tumor shows bone destruction and a "myeloma" kidney. (3) The lactogenic effect is more extreme in one subline.

Significance to Cancer Research: Neoplastic alterations in cells which have easily recognized morphologic or metabolic characters may lead to a clearer understanding of neoplasia.

Proposed Course of Project: It is expected that publications describing these three neoplasms will be completed within the next year. A number of other neoplasms are being carried in transplant, and others are being obtained. Descriptions of these will be published if any prove to be of unusual interest.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 506
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$10,550	$4,190	$14,740	1/3	1	1-1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1	1 1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16, or 17

(Use reverse and additional pages, if necessary)

Form No. CA-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 507
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Mitochondrial Changes in the Reticulo-endothelial System of x-irradiated Mice and Protected Against Radiation Sickness by Intravenous Injection of Bone-marrow.
PROJECT TITLE

7. Ross C. MacCardle
PRINCIPAL INVESTIGATOR

8. Charles C. Congdon
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine whether the mitochondria constitute a focus of x-radiation injury to reticulo-endothelium, and whether the protective property of bone-marrow may involve repair of the chondriome.

Methods Employed: We x-irradiated mice and treated them with bone-marrow in the usual way, with adequate controls without bone-marrow. We also treated mice with lethal doses of sodium azide (a powerful inhibitor of cytochrome oxidase, an enzyme residing in the mitochondria according to Hogeboom & Schneider) to determine whether bone-marrow may also protect against such chemical injury inflicted by sodium azide.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Major Findings: The results of these experiments so far indicate that the mitochondria are altered and diminished following x-radiation, and that treatment with bone-marrow in most cases prevented the damage, at least in degree. So far, sodium azide was found to have no morphologically detectable effect on the mitochondria in the early stages of treatment. It was only after the late moribund state had obtained when the mice were nearly dead that a definitive effect of Na azide was found.

Significance to Cancer Research: X-radiation can induce cancer, and it is possible that its carcinogenic action may be mediated through the mitochondria.

Proposed Course of Project: We have already begun a study of the liver of mice in which the whole body was irradiated while the liver was shielded, and of liver only x-irradiated with the rest of the body shielded.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 507
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,950	$1,570	$5,520	1/2	0	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	0	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16 or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 508
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Comparative Study of Normal Pilosebaceous Apparatus in Human Skin and those of Basal-cell Tumors and of Acne.
PROJECT TITLE

7. Dr. E. J. Van Scott and Ross C. MacCardle
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

This project is being reported by Dr. Van Scott and a full paper on the subject is in press - Journal of Investigative Dermatology, 1956.

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 508
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,950	$1,560	$5,510	1/2	0	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	0	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16 or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 509 A
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Cytological Origin of Thyrotrophin-secreting Tumors of the Anterior Lobe of the Pituitary Gland of Radiothyroidectomized Mice, and of Human Pituitary Tumors.
PROJECT TITLE

7. Ross C. MacCardle
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The object of this study is to ascertain and define the earliest morphological alterations that occur in cells of tissues in their change from the normal to the malignant state of protoplasm in respect to a possible analysis of the underlying cause of the malignant transformation. This tumor of the mouse represents one of the most valuable experimental tumors because it resembles pituitary tumors of man. It clearly consists of a single, histologically-identifiable type of tissue. One or another of the constituent cells of the anterior lobe proliferate in different states of hyperplasia and malignancy. The gland is stimulated to become hyperactive in producing the thyroid-stimulating hormone for a thyroid gland that has been essentially destroyed by treatment with radioiodine. Finally, the

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

hyperplastic tissue becomes malignant to produce 10 times as much hormone as the normal gland. The amount and kind of hormone produced by a tumor can be determined, and the cytological alterations of the same tumor can be identified and related to the function.

Methods Employed: Cytological methods can detect alterations in cellular minerals (microincineration), mitochondria, Golgi apparatus, nucleoproteins, cytochrome oxidase granules.

Major Findings: Most of the cells of the thyroidectomy-dependent tumor appear to derive from alpha cells, while about 50% of the autonomous (independent) tumor seems to derive from alpha cells and the other half from beta cells (although in the normal gland it is very difficult to identify beta cells for comparison with tumor cells).

Proposed Course of Project: The proposed course of the project is to study the fetal, adult and hyperplastic gland to compare the relation of cytological changes to production of hormones with those of primary and transplant malignant tumors. It is clear that a study of the effect of TSH on the cells of an already developed TSH-tumor should be made to determine the cellular organoids first affected when the activity of the tumor begins to diminish in the presence of TSH. Comparative cytological studies of human and animal pituitary tumors should be accomplished. It is proposed to study the effect of radioiodine on young and adult dwarf homozygous mice that lack pituitary alpha cells.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 509 A
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,950	$1,570	$5,520	0	1/2	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	1/2	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16 or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 509 B
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. "The Tumor Cell: Characteristics of Malignant Protoplasm" as part of a series of volumes of an International Treatise on Physiology of Protoplasm "Handbuch der Protoplasmaforschung" being edited by Heilbrunn and Weber.
PROJECT TITLE

7. Ross C. MacCardle
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The object of this monograph is to summarize and correlate our knowledge of the properties and possible identification of a malignant cell of a tumor.

Methods Employed: By reviewing the literature and relating some new data found by the author, it is clear that although a single white blood cell of the

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Major Findings: circulating blood of a leukemic mouse can upon injection into a normal host produce leukemia (notwithstanding the serum injected with the cell), such a cell possesses no anatomical features by which it can be distinguished from its normal cell of origin. The most malignant tumor constitutes several types of cells, some of which may have nothing to do with malignancy and none of which can be detected as a malignant cell. The transplantability of the malignant tumor cells is discussed, and the cytology of several tumors is described for the first time.

Significance to Cancer Research: This paper will bring together old and new data in an effort to evaluate the status of our knowledge of the mechanism of the transformation of normal protoplasm to malignant protoplasm.

Proposed Course of Project: This monograph is now completed in long-hand ready to be typed to be submitted to the printers. It is scheduled for final printing and publication in March 1957.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 509 B
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,950	$1,560	$5,510	0	1/2	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	1/2	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16 or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 509 C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Intracellular Cytology of Human Tumors and of Animal Tumors Transplanted in Castrated Hosts.
PROJECT TITLE

7. Ross C. MacCardle
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: Little is known of the basic intracellular cytology of the cytoplasm of human and animal tumors under

Methods Employed: I have microincinerated a large number of human and animal tumors and compared the mineral pattern of the tumor with the amount and location of cytoplasmic ribonucleic acid. I have also studied this relationship in transplantable animal tumors at the early and late stages of transplantation.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Major Findings: It is clear that rapidly-growing animal tumors contain relatively less calcium/magnesium ash and less cytoplasmic ribonucleic acid than slowly-growing tumors. The mineral pattern of human tumors varies.

Proposed Course of Project: I have prepared a manuscript showing the ash-pattern of several human tumors as related to Cytoplasmic Ribonucleic acid. I have also described the cytology of Sarcoma 37 of mice during the early and late stages of its implantation in castrated and normal male and female hosts. I propose to determine the effect of adrenalectomy on the growth and cytological form of Sarcoma 37 in respect to imbalance of sodium and potassium.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 509 C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,950	$1,570	$5,520	0	1/2	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	1/2	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16 or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 510
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Study of Endocrine and Reproductive Organs in the Mouse.
PROJECT TITLE

7. Dr. Thelma B. Dunn
PRINCIPAL INVESTIGATOR

8. Dr. Ross MacCardle is doing some phases of this study.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To accumulate reliable data by observations on untreated mice, planned procedures, and reviews of the literature regarding the reproductive and endocrine organs of inbred mice.

Methods Employed: Personal observations on material submitted from different sources, planned experimental procedures in inbred mice, histologic study of untreated mice at various ages, systematic review of the literature.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Major Findings: This study is proceeding slowly because of pressure of other work. Considerable morphologic difference is found in the adrenal glands of inbred strains of mice.

Significance to Cancer Research: Since hormonal studies are an important part of cancer research, it is desirable that reliable information be available regarding the endocrine and reproductive organs of inbred strains of mice.

Proposed Course of Project: It is expected that this study will proceed slowly, and require several years for completion.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 510
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$10,550	$4,200	$14,750	1/3	1	1-1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1	1 1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16, or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 511
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Steroid Studies in Stressed and Tumor-bearing Animals and Man.
PROJECT TITLE

7. Dr. Eli M. Nadel
PRINCIPAL INVESTIGATOR

8. Mr. Bobby G. Young and Mr. Arthur Hilgar
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To quantitatively measure the polar corticosteroids present in the blood, urine, and tissues of man and animals (normal, stressed, and tumor-bearing) and to correlate where possible the clinical course with the adrenal and other morphologic alterations and with specific corticosteroid excretion patterns.

Methods Employed: The term stress is used in its widest concept for the purposes of this report and includes dietary deficiency and excesses as stresses, hormonal excesses, and deficiencies as stresses, x-ray irradiation effects, stress by physical and microbial agents, carcinogens, and the effect of carcinoclastic and potentially carcinostatic drugs.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Animals: Stressed animals, normal controls and tumor-bearing animals are prepared biologically and the clinical course followed. Tissues, blood, and urine are collected during the course of the observation period and at post-mortem examination, for chemical, histochemical, and histopathological study. Extraction, purification and analysis are done by specific applications of chromatographic and spectrophotometric methods.

Man: Clinical patients from the Leukemia Service, NCI, Surgical Service, NCI, and Psychosomatic Service, NIMH, are being studied in addition to non-patient controls. Analyses for polar corticosteroids are performed on the urine of patients before, during, and after the administration of ACTH.

Major Findings: 1. A rapid test for thymolytic activity of corticoids in nestling rats has been developed. Two workers (working 3 hr. each) can conveniently compare in a 24-hr. period the thymolytic activity of an unknown with that of cortisol and controls without the necessity of adrenalectomy, postsurgical care or special diets. The semilog-dose; response of cortisol is linear. By this test metacorten had 3-4 times the activity of cortisol. Presented Fed. Proc. vol. 15, March 1956.

2. There is increased incorporation of radio-acetate into adrenal cortisol and corticosterone in scurvy as measured by direct isolation studies (Dr. Burstein) and by chromatographic identification studies (Mr. Young and Dr. Nadel). Presented Fed. Proc. vol. 15, March 1956.

3. The value of ethylacetate extraction procedures over that using less polar solvents originally suggested by the Ciba group in Switzerland and confirmed by Meyer and later by Burstein at Worcester Foundation has been applied to extraction of urine in man and animals with the isolation and identification of 2 new hydroxylated derivatives of cortisol, 6β-hydrocortisol and 2 α-hydroxycortisol.

4. Two tumors in inbred guinea pigs have been maintained for the past five years and utilized in steroid studies.

5. Steroid excretion studies in neoplastic disease in man were presented at the Am. Fed. Clin. Res. in New Orleans in January 1956.

10. PROJECT DESCRIPTION (Cont'd)

Significance to Cancer Research: The presence of compounds, more polar than cortisol in the urine of man and of quinea pigs, indicates: (1) Conclusions based on the excretion of corticosteroids without specific reference to their identity must be re-evaluated, e.g. the principle corticosteroid in the albino Hartley guinea pig is cortisol, while the principle corticosteroid in Strain 2 and Strain 13 guinea pigs is not cortisol. (2) When hydrolyzed urines are extracted and their glycogen potency compared with that of extracted unhydrolyzed urine there is little difference in the biologic activity of the 2 extracts. Inasmuch as the free unconjugated polar urinary corticosteroids are the biologically active materials responsible for nearly all of the glycogenic activity of hydrolyzed or unhydrolyzed urine it now becomes important to study these free corticosteroids in some detail. (3) Up to the present, insufficient attention had been given to these new polar corticosteroids for 2 reasons: a) they were not known to be present prior to their recent discovery by us in the urine of guinea pigs and of man; and b) previous methods of extraction actually destroyed them.

Proposed Course of Project: The various data that have been accumulated within the past five years (on this general and broad subject) in this laboratory will be prepared for publication as time permits but no new projects or continuation are contemplated.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 511
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$15,790	$6,280	$22,070	1/2	1-1/2	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	1 1/2	2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 511
Serial Number

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Nadel, E. M., Burstein, S., and Dorfman, R. I. Isolation of Polar Reducing Corticosteroids from Human Urine. Archives of Biochemistry and Biophysics, 61 (1): 144-149, March, 1956.

Taylor, D. J., Greenberg, J., Josephson, E. S., and Nadel, E. M. Histochemical Sudanophilia and Cholesterol Concentration in the Adrenal Glands of Chicks Parasitized with Plasmodium Gallinaceum. Acta Endocrinologica 22: 173-178, 1956.

Nadel, E. M., and Haas, V. H. Effect of the Virus of Lymphocytic Choriomeningitis on the Course of Leukemia in Guinea Pigs and Mice. J. Nat. Cancer Inst., 17: 221-231, August, 1956.

Nadel, E. M., and Burstein, S. Urinary Excretion of Corticosteroids in Guinea Pigs with Malignant Neoplastic Diseases. J. Nat. Cancer Inst., 17: 213-220, August, 1956.

Nadel, E. M., and Hilgar, A. G. Rapid Test for Thymolytic Activity of Corticoids in Nestling Rats. Fed. Proc., 15: No. 1, March, 1956.

Burstein, S., and Nadel, E. M. Increased Incorporation of Radioacetate into Adrenal Cortisol and Corticosterone in Scurvy. Fed. Proc., 15: No. 1, March, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 51h
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. A Study of the Transplantable Adrenocortical Adenocarcinoma in Osborne-Mendel Rat - Steroids Synthesized; Their Physiological effects on the Host; Metabolism of the Neoplasm; and Responses of the Neoplasm to Hormones and Therapeutic Agents.
PROJECT TITLE

7. Dr. A. S. Mulay
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: Physiological, chemical, and metabolic definition of the steroids of the adrenocortical adenocarcinoma by isolation and identification of the adrenocortical steroids of the adenocarcinoma; Definition of the conditions for the synthesis of these steroids by the neoplasm; Physiological effects of the growth of the neoplasm on the host; Effects of gonadotropins and drugs on the growth and activity of the adenocarcinoma.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Methods Employed: Transplanting the adenocarcinoma to intact, gonadectomized, or adrenalectomized rats, which are untreated, or treated either with hormones or with drugs. Induction and growth of the adenocarcinoma in hosts of different strains and observing the physiological effect of the tumor growth on the host and of hormones and drugs on the neoplasm. Steroid extraction, purification, isolation, and identification on tumors harvested and stored in deep freeze for this purpose.

Major Findings: (1) This adrenocortical adenocarcinoma maintains adrenalectomized Osborne-Mendel host in good health without any sodium chloride or adrenocortical therapy. (2) Urine-sugar output of the host rat bearing this neoplasm, seems to be higher than that of the corresponding controls. However, there is no significant difference between blood-sugars of the host rats and the controls. (3) Growth of this adenocarcinoma in Osborne-Mendel host results in an increase in the weight of spleen and kidney and a reduction in the weight of adrenals and gonads in the hosts of both sexes and an increase in the weight of the heart of the male hosts. (4) Amphenone (single dose, divided dose, or continuous drip) treatment shows a marked effect on the adrenals and thyroid of the host, however, no clear-cut effect has been observed on the neoplasm. (5) This adenocarcinoma has been successfully adapted to M-520 rats from the original Osborne-Mendel rats. In response to the growth of this neoplasm, adrenals of the host are reduced to about 7 mg. or less each, in place of their normal 15-20 mg. weight.

Significance to Cancer Research: Definition of the steroids synthesized by this neoplasm, and their relation to those synthesized by normal adrenal cortex, may give us some insight into the changes which transform normal adrenal gland into adrenocortical adenocarcinoma.

Proposed Course of Project: Chemical and metabolic studies of this tumor are still in their preliminary stages and it will take next calendar year before some results begin to come out. Growing of the tumors to collect the material, extraction, purification, isolation, and identification are tedious tasks which take time.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 514
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,850	$4,720	$16,570	1/2	1	1-1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	1	1 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 514
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Symeonidis, A., and Mulay, A. S. Histopathology of adrenal glands of rats fed a low-protein, low-riboflavin diet, alone or with para-Dimethylaminoazobenzene. J. Nat. Cancer Inst. 16: 1163-1179, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 515
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Relation of Hormonal Factors to the Induction of Adrenocortical Tumors in Osborne-Mendel Rats.
PROJECT TITLE

7. Dr. A. S. Mulay
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine the role played by hormones on the induction of adrenal tumors in Osborne-Mendel rats.

Methods Employed: Intact or gonadectomized rats, with or without hormone implants are kept on low protein, low vitamin, semisynthetic diet with or without the addition of azo dye carcinogen to it. Animals are examined for tumor development at regular intervals. Adrenal glands of the rats sacrificed for study are processed for chemical and microscopic examination.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Major Findings: In the study of intact male animals, eighty percent of the rats examined thus far (36 rats) show an enlargement of either one or both of the adrenal glands. Pieces from one such adrenal gland, weighing over one gram, were successfully transplanted to Osborne-Mendel rats. Chemistry and pathology of these adrenal glands are under study. Experiments on other phases of this problem are just starting.

Significance to Cancer Research: Evidence thus gathered may throw some light on the factor or factors in part responsible for the induction of adrenal neoplasms.

Proposed Course of Project: Experiments on some phases of this experiment will start as time, space, and materials permit.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 515
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,850	$4,720	$16,570	1/2	1	1-1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	1	1 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16, or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 516
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Induction, Pathogenesis and Morphological Alteration of Tumors Arising in Bone.
PROJECT TITLE

7. Dr. Albert W. Hilberg
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The recognition and evaluation of the various tumors arising in bone in the laboratory animal in order to determine the significance of these changes and their subsequent behavior under various conditions as may be produced in the laboratory. This involves not only spontaneous tumors but also the tumors of bone which may be induced in the animal.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Methods Employed: Animals bearing spontaneous tumors of bone are autopsied and their bone tissues studied histologically. The vital tissues of the animal are also studied in detail in order to detect any changes which may have occurred.

Spontaneous tumors of bone are maintained by serial transplants to succeeding generations and the changes assessed by histologic examination of tissues obtained by autopsy. Transplants of the tumors are made in various body sites to study the differences in morphology which may result and to determine the possible significance. Tumors for this study are obtained from various laboratories in the National Cancer Institute.

Major Findings: To date more than forty spontaneous tumors of bone in the mouse have been studied and the morphological details of the tumors and tissues have been tabulated for evaluation. Of these tumors six have been successfully carried in transplant for several generations.

The beryllium injections of rabbits and mice have been completed and almost all of the animals involved have been autopsied. Among the fifty rabbits given a single injection of beryllium oxide there have been three tumors. Two of these are bone tumors and one is a lymphoma. Spleens and livers of these animals have shown peculiar degenerative changes and atrophy.

Morphologic variation of spontaneous bone tumors carried in transplant into the kidney area have shown a distinct change from anaplasia to a greater differentiation to bone growth in the tumor tissue. (See publications, Part C, 16.)

Beryllium injections of mice are complete and to date one bone tumor has been found in a C57 Black mouse.

Significance to Cancer Research: Information regarding the morphology and development of bone tumors in laboratory animals and the alterations of these tumors that may be produced by transplanting in different body sites may give some clue to the understanding of the factors which produce these tumors and why these tumors vary in their progression and prognosis.

The evidence of tumor production from minimal single doses of beryllium compounds is of value because of the implications to the human problem in the beryllium industries and resultant exposure.

10. PROJECT DESCRIPTION (Cont'd)

Proposed Course of Project: The continued observation and evaluation of the morphology of spontaneous bone tumors in the laboratory animal. The continued study of alterations in morphology that can be produced by selected site transplantation.

The complete evaluation of the results of beryllium injection of rabbits and mice and the subsequent publication of the significant results and findings of the study.

The continued consultation work with various laboratories of the National Cancer Institute and work as a consultant to the Argonne National Laboratory of the Atomic Energy Commission.

The investigator spends most of the time as Head, Cytodiagnosis Section, Pathologic Anatomy Branch.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 516
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,850	$4,720	$16,570	-	1-1/2	1-1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	1 1/2	1 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 516
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Hilberg, A. W. Morphologic variations of an osteogenic sarcoma of the mouse when transplanted to the kidney. J. Nat. Cancer Inst. February 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 517
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Pathological Technology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Pathological Technology Section
PROJECT TITLE

7. Mr. J. M. Albrecht
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Stained tissue sections are the fundamental basis of all clinical and experimental studies of cancer. The Section prepares histological sections for all the investigators of the National Cancer Institute. It makes available all the established routine and special stains and in addition develops and provides the current experimental methods of tissue preparation such as enzyme stains and specific histological stains.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

November 1, 1955 - November 1, 1956

Number of Investigators	62
Number of Pieces of tissue	119,874
Number of Bottles of tissue	19,529
Number of Blocks cut	63,294
Blocks cut serially	614
Frozen blocks cut	1,402
Number of autopsies recorded	14,592
Number of recuts	1,170
Number of slides stained H & E	73,211
Number of slides stained special	24,036
Number of unstained slides	11,325

Photographic Service rendered to the Laboratory of Pathology, November 1, 1955 to November 1, 1956.

GROSS

Black & White	61
Color	23

MICROS

Black & White	703
Color	894

LANTERN SLIDES

Black & White	134
Color	612

PRINTS

4 x 5	2099
8 x 10	51
Mounted	183

Form No. CRP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 517
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$140,660	$46,350	$187,010	3	22-1/2	25-1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3	22 1/2	25 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 517
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

Pathological Technology Section received a Group Award for outstanding work performance on August 20, 1956.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 518
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Cytochemistry and Cytopathogenesis of Cutaneous Lesions of Psoriasis, including a Description of Golgi Apparatus, Minerals and Ribonucleic Acid of Normal Human Skin.
PROJECT TITLE

7. Ross C. MacCardle
PRINCIPAL INVESTIGATOR

8. Dr. Richard Reinertson
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Dr. Reinertson is reporting this project. He and I have two manuscripts in preparation on this study.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 518
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,950	$1,560	$5,510	0	1/2	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	1/2	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16 or 17

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 519
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Study of the Chemical and Histopathological Changes induced in Adrenal Glands of Rats Fed Diets Deficient in Proteins (Amino Acids) and Vitamins like Riboflavin and Pteroylglutamic Acid.
PROJECT TITLE

7. Dr. A. S. Mulay
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine deficiency or deficiencies which induce histopathological changes in adrenal glands of rats, time of their induction, and changes in the chemistry of the adrenal gland concomitant with the histopathological changes.

Methods Employed: Complete and deficient synthetic diets of known composition are fed to groups of male and female rats. Animals are sacrificed from each group at regular time intervals and adrenals are processed for histological and chemical examination. Determination of ascorbic acid, cholesterol, esterases, and adenosine triphosphatase are undertaken.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Major Findings: Histopathology of the adrenal glands of male Osborne-Mendel rats on deficiency diet for 6 months or more is reported in a preliminary paper in the J. Nat. Cancer Inst. 16: 1163-1179, 1956. Current studies reveal that these changes in adrenal glands of rats are grossly visible three months after the start of the deficiency diet. Adrenal glands of these animals are smaller in size and weight, very soft, and have a higher ratio of cortical to medullary area. Adrenal glands of female rats on deficiency diet show changes similar to those in males similarly treated.

Significance to Cancer Research: Knowledge of the deficiency or deficiencies which bring about the histological changes in the adrenal glands and the knowledge of the chemical and metabolic changes related to this transformation, will bring us a step nearer to understanding the role played by the adrenal glands in the neoplastic process in general and induction of hepatomas by azo dye carcinogens in particular.

Proposed Course of Project: Histological and chemical examination of the adrenal glands of the rats on experimental diet for 3 months is in progress. Other experiments are in different stages of planning and execution.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 519
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,950	$1,570	$5,520	0	1/2	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	1/2	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16, or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 520
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Tissue culture of mouse leukemias.
PROJECT TITLE

7. Dr. Clyde J. Dawe
PRINCIPAL INVESTIGATOR

8. Dr. Michael Potter
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The isolation and continuous cultivation in vitro of the various types of leukemia cells of mice.

Methods Employed: Methylcholanthrene-induced leukemias transplantable in ascites form in DBA or $DBAF_1$ hybrid mice are used as sources of cells. The cell types of leukemias now available include granulocytic, lymphocytic, plasma cell, mast cell, and reticulum cell sarcoma. Various tissue culture methods and media are used in an attempt to determine the most favorable circumstances for cell survival and proliferation. Alternate passage of cells through mice and tissue culture is being carried out to determine whether this may favor "adaptation" of leukemia cells to tissue culture conditions.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

The survival time of leukemia cells in cultures is determined by direct observation and by inoculation of mice with cultures at various time intervals.

Major Findings: Two cell lines have been isolated and show promise of continuous in vitro propagation. One is a histiocytic subtype of reticulum cell sarcoma, designated P329, which has been cultivated continuously on glass in roller and flat tubes for 10 months. This cell is highly phagocytic, closely resembles its normal analogue, the macrophage, and thus far continues to produce neoplasms true to type when inoculated back into mice.

The second cell line was originally classified as lymphocytic neoplasm (P388), but in the course of adapting to tissue culture maintenance it underwent a morphologic transformation to a larger cell more closely resembling the reticulum cell. This cell line has now been carried continuously for nearly 4 months in tissue culture, and is beginning to show evidence of losing its tumor-forming ability. In the early passages of the transformed cell, tumors were producible in mice, and these showed morphologic aberrations from the original lymphoid neoplasm consistent with the changes of the cells in vitro.

In the alternate passage experiment, it has been found that alternate tissue culture-to-animal passage does not enhance the ability of the cells to grow in vitro. On the contrary, repeated animal-to-animal passage did lead to cell lines with increased survival time in vitro.

Significance to Cancer Research: The isolation of these cell lines provides material for studies of growth characteristics, nutritional requirements, and susceptibility of leukemia cells to therapeutic agents in a relatively controlled environment. By study of the morphologic characteristics of leukemia cells continuously carried in vitro, it may become possible to explain some hitherto puzzling morphologic pictures seen in both man and mouse in vivo.

Proposed Course of the Project: To continue attempts to cultivate the various cell types of leukemia, until at least one example of each type can be carried. The already isolated lines will be studied for further morphologic and functional alteration as they are carried over longer periods of time.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 520
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,850	$4,720	$16,570	1/2	1	1-1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16 or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 521
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Transformation of Leukemia Cells in vitro.
PROJECT TITLE

7. Dr. Clyde J. Dawe
PRINCIPAL INVESTIGATOR

8. Dr. Michael Potter, Dr. William G. Banfield
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To observe the morphologic steps involved in, and the factors influencing the transformation of leukemia cells in tissue culture.

Methods Employed: A lymphocytic neoplasm has been observed to undergo morphologic transformation to a reticulum cell sarcoma while in tissue culture. To characterize as accurately as possible the sequence and nature of the changes in the cell, the following methods will be used:

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

1. Direct observation of transformation, using time-lapse cinematography with phase optics.

2. Cytochemical and supra-vital staining studies of the transformed cell and its prototype.

3. Electron microscopy of the transformed cell and its prototype.

4. Chromosome studies of the transformed cell and its prototype, in conjunction with transplantability studies.

Factors possibly influencing the transformation phenomenon will be sought by observing effects of altering gas tensions, pH, temperature, serum concentration, and various nutrient components of the medium.

Major Findings: This work is now in an early stage. The observation of a transformation has been repeated once, and second and third repeat experiments are in progress. In the two instances observed, the derived cell lines are very similar to one another, both resembling reticulum cells. Many multinucleated forms are present, and preliminary chromosome counts indicate a sub-tetraploid number in the new line as compared to a diploid number in the original. The new lines in both instances show increased ability to survive in vitro, and in the one line studied with respect to phagocytosis, this activity is increased over the prototype. The tumors produced by the transformed cell lines kill the host animals less rapidly than does the original cell, but the ascites-producing capacity is retained. The possibility that the transformed line is a "de novo" tumor derived from a host during passage has been ruled out by transplantation studies showing the derived line to be DBA/2 in origin, while passages were carried out only in CXDBA/2 hybrids. A comparison of drug sensitivities of the new and old lines shows the two to be similar in this respect.

Significance to Cancer Research: Morphologic mixtures and variations within individual cases of malignant lymphoma and leukemia have been observed for years. Study of the above system may provide information on the mechanism and origin of such variations. More important, probably, is the possibility of correlating morphologic characters with the genetic constitution of cells in such isologous pairs. It is hoped this system will prove useful in providing evidence for or against the transfer of genetic materials from one cell to another.

10. PROJECT DESCRIPTION (Cont'd)

Proposed Course of Project: Information gained from the above experiments will be used to attempt induction of transformations in other leukemia cell lines. The possibility of utilizing transformed lines for induction of immunity is under consideration, but preliminary experiments are necessary before placing any emphasis in this direction.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 521
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,900	$3,150	$11,050	1/2	1/2	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	1/2	1	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSIST-ANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16, or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 522
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. An Investigation of the Collagenous Connective Tissues.
PROJECT TITLE

7. Dr. William G. Banfield
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The immediate objectives are:

1. To elucidate the age changes in collagen.

2. To uncover mechanisms affecting the usual state of the collagen in the body.

Methods Employed:

1. Collagen from human skin, scars and Achilles tendons are subjected to dilute acetic acid in order to establish more definitely the normal limits for the age-swelling pattern

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

of Achilles tendon and to measure the amount of soluble collagen present in these tissues. Variations in the reaction of skin and Achilles tendon from the normal are investigated through the patients history and possible causes of such variation are subject to animal test. The maturation of scars is studied with respect to changes in acid-soluble collagen and histology.

2. Hamsters are tested for changes in skin collagen after hormone treatments or gland removal.

3. Basic fuchsin was tested as a stain for differentiating young and old collagen.

Major Findings:

1. The decrease in concentration of acetic acid-soluble collagen in human skin at one year of age and again above 30 years of age has been confirmed.

2. Abdominal skin from surgical biopsies contains a higher concentration of acetic acid-soluble collagen than abdominal skin from autopsies. This difference seems to occur before death.

3. The present data indicate that there is a decrease in the concentration of acetic acid-soluble collagen of scars at about one year after wounding.

4. The swelling of human Achilles tendon increases from birth until approximately 26 years of age. After 26 years of age the acid swelling capacity decreases until the age of 60, following which there is very little swelling. A chart of this data has been published.

5. Basic fuchsin so far has not proved practical for differentiating old from young collagen.

6. There is a strong indication that old hamsters treated with growth hormone have a higher concentration of acetic acid-soluble collagen in their skin, than do the untreated hamsters.

10. PROJECT DESCRIPTION (Cont'd)

Significance to Cancer Research: If the results with the growth hormone prove to be valid, it would indicate that the measurement of concentration of acetic acid-soluble collagen in a tissue is a measure of one aspect of the collagen metabolism in that tissue and this metabolism can be altered by one hormone, at least, and very likely by other means. The implications of such a finding and the development of simple methods for measuring collagen metabolism would have far-reaching applications in the study of cancer as well as in many other fields of medical research with the opportunity of applying the techniques in human clinical investigations.

The results with the scars are similar to the results obtained from autopsy skin, that is to say, there is a decrease in the acetic acid-soluble collagen at one year. This would suggest that similar factors are acting in both instances. This gives a basis for speculation on a growth process and its normal control and suggests new methods of approach to the investigation of some growth processes.

Proposed Course of Project:

1. Chemical and physical chemical methods, which for the most part are available, should be worked out for determining the acetic acid-soluble, neutral salt soluble, and possibly other fractions of collagen in this laboratory.

2. These should be used in conjunction with the present method of estimating acetic acid-soluble collagen in a definitive investigation.

   (a) To ascertain the normal age changes in the collagen of the skin of hamsters.

   (b) To reassess the effect of growth hormone on collagen metabolism.

3. Experiments should be set up using N^{15} labeled glycine to determine exactly what the concentration of acetic acid-soluble collagen in a tissue means.

4. Hamsters should be tested for acetic acid-soluble collagen in the skin after various endocrine gland removals, starvation, etc.

10. PROJECT DESCRIPTION (Cont'd)

5. Biopsies of abdominal skin from patients on long term hormone or antimetabolite therapy or after gland removal, such as hypophysectomy, should be taken before and after and tested for acetic acid-soluble collagen.

6. More data on the acetic acid-soluble collagen in scars up to two years of age will be accumulated.

Form No. ORP-1
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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 522
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$15,790	$6,280	$22,070	1/2	1-1/2	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	1 1/2	2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16, or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 523
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Effects of Ionizing Radiation with Emphasis on the Morphologic Alterations and Neoplasms.
PROJECT TITLE

7. Dr. Richard L. Swarm
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The collection of additional information regarding the development of neoplasms in man and laboratory animals following exposure to ionizing radiation is one general objective of this study. Other changes produced by ionizing radiation are also the object of the study. The differences in the biologic effects of different types of ionizing radiation is a point of principal interest. The localization and biologic effect of several different isotopes are to be studied. A knowledge of some of the changes effected by different types of radiation in existing tumors will be sought.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Methods Employed: The approach to this study will include continuation of the following activities:

1. Participation in the activities of the Pathologic Anatomy Department of the Clinical Center. Here a review of accessioned specimens is made possible by actual participation in the study and diagnosis of specimens as they are received. Special emphasis is made in the study of material demonstrating radiation change or containing radioactive isotopes.

2. Detection of neoplasms and other morphologic changes in experimental animals who have received external x-irradiation or internally administered radioactive isotopes.

3. A study of the distribution and effects of radioactive isotopes of several types. Here the emphasis is on the utilization of gross and microscopic autoradiographic techniques combined with more conventional gross and histologic techniques.

4. To facilitate the above and to assist other investigators collaboration on problems in several fields is in progress.

(a) With Dr. W. W. Smith; the study of animals which have survived the administration of large amounts of external radiation.

(b) With Drs. Rubin, Brace, and Andrews, et al; the study of animals given therapeutic amounts of radioactive sulphur.

(c) With Drs. Brace, Rubin, Andrews, et al; the study of the effects in cartilage in animals given localized external radiation to the extremities.

(d) With Drs. Schatten, Bergenstal, et al; the study of cartilage and transplanted cartilage utilizing radioactive sulphur.

(e) With Dr. H. Levine; the study of zinc and zu 65 in leukocytes and certain tissues.

(f) With Dr. H. Morris; the study of the effects of endocrine glands on the occurrence of hepatic neoplasms induced by 2 diacetylaminofluorine.

(g) With Dr. Schatten, et al; the study of transplants to the extra hepatic bile ducts in dogs.

10. PROJECT DESCRIPTION (Cont'd)

(h) With Drs. D. Rall, T. L. Loo, Dr. T. Dunn, et al; the study of normal and tumor tissue from animals given tetracycline.

5. A study of the distribution and effects of internally administered colloidal thorium dioxide has been continued by: (1) the study of human case material at the Clinical Center and the Armed Forces Institute of Pathology and (2) the administration of this material to experimental animals.

Proposed Course of Project: (1) The activities outlined above under methods are to be continued. (2) Emphasis on the study of the biologic distribution and effects of radioactive isotopes of different types will continue. Particularly of interest will be the study of S^{35} in cartilage and of thorium dioxide in the reticulo endothelial system. Study of other isotopes as the opportunity for collaboration presents itself is intended. (3) To facilitate better utilization of radioisotopes in this study the following are recommended:

1. Some special training for Dr. Swarm

2. Purchase of some specialized equipment

3. Improvements in dark room facilities

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 523
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$23,690	$9,440	$33,130	1	2	3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	2	3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 523
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

Dr. Richard L. Swarm was certified in Pathologic Anatomy by the American Board of Pathology.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 524
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Pathology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Morphological characteristics of human neoplasms in tissue culture.
PROJECT TITLE

7. Dr. Alan S. Rabson
PRINCIPAL INVESTIGATOR

8. Miss Frances Legallais
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The principal objective of this study is the exploration of possible diagnostic and prognostic applications of the tissue culture method to the study of human neoplasms from the standpoint of the surgical pathologist. The identification and cultivation of a variety of human neoplasms in vitro would, of course, also be of considerable value in studies of oncolytic viruses and chemotherapeutic agents.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Methods Employed: Explants of tumors removed at surgery in the Clinical Center are grown in plasma clot on cover-slips in Leighton tubes. Media containing large amounts of human serum as well as a variety of amino acids and other constituents are used. At appropriate intervals, cultures are fixed and stained for study. The basic fixation and staining technique used has been that of Papanicolaou since a large body of information on the identification and characterization of malignant cells with this procedure has already been accumulated. Other staining techniques have been employed when indicated.

Patient Material: Cultures of tumors from approximately 50 patients have been studied since this project was begun in June of 1956. These have included 14 epidermoid carcinomas from various primary sites, 2 basal cell carcinomas, 5 malignant melanomas, 2 thyroid gland carcinomas, 1 adrenal gland cortical carcinoma, 2 ganglioneuromas, 2 carcinomas of the breast, 1 malignant cystosarcoma phyllodes of the breast, 1 leimyosarcoma of the vagina, 1 meningioma, and a variety of other neoplasms and normal tissues.

Major Findings: As noted above, only a relatively small number of neoplasms have been examined, and it would be inappropriate to draw any conclusions from the material available at the present time. One observation which may seem obvious has been the extreme importance of proper selection of the specimen used for tissue culture. Too often, the surgical pathologist has difficulty in definitely identifying tumor grossly and the material obtained for cultures is composed of inflammatory tissue and fibrous tissue with few or no tumor cells present. If the sample obtained for culture consists primarily of tumor tissue with a minimum of stroma, satisfactory outgrowth at least for short periods can be quite consistently obtained with epidermoid carcinomas.

Significance of Project to Program of Institute: The utilization of morphological characteristics of human neoplasms in tissue culture in diagnosis and classification might be of considerable value in the surgical pathology activities of the Pathological Anatomy Branch of the National Cancer Institute. As noted above, the availability of a variety of human neoplasms in culture would be of value in the adaptation of oncolytic viruses to specific tumors and might also be advantageous in chemotherapeutic studies.

10. PROJECT DESCRIPTION (Cont'd)

Proposed Course of Project: The present plans are to continue tissue culture studies of human tumors as they become available in the surgical pathology laboratory of the Pathological Anatomy Branch. It is hoped that with increased experience with a variety of neoplasms, cultural characteristics and growth patterns of diagnostic and prognostic value may be observed.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 524
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$15,790	$6,280	$22,070	1	1	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16, or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 525
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Attempt to induce carcinoma of the bladder in hamsters by infection with Schistosoma haematobium.
PROJECT TITLE

7. Dr. Eloise Cram, Dr. Louis B. Thomas, Dr. Elmer Berry and Dr. Louis Olivier
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

Not to my knowledge.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

This research project is in collaboration with Doctors Eloise Cram, Elmer Berry and Louis Olivier N.I.A.I.D., and consists of studying chronic infection with S. haematobium (Gold Coast strain and Egyptian strain) in hamsters; particularly with reference to the induction of bladder carcinoma.

a. 217 hamsters were exposed to approximately 300 cercariae each early in 1955.

b. Approximately 90 of these infected hamsters are still living and will be allowed to live as long as possible to study the effects of chronic infection with S. haematobium.

(Use reverse and additional pages, if necessary)

c. During 1956 S. haematobium (Egypt) cercariae have become available and to date approximately 30 animals have been exposed to approximately 300 cercariae each. Thus, when this chronic experiment is terminated it will be possible to study chronic infection of S. haematobium obtained from two different areas of Africa, namely, Egypt and the Gold Coast.

Significance to Cancer Research:

S. haematobium infection has been found associated with bladder carcinoma in several parts of the world. This study is designed to produce bladder cancer in the hamster by chronic infection with S. haematobium. The incidence of bladder cancer associated with Schistosomiasis is high in Egypt and relatively low in the Gold Coast. The study is also designed to determine whether there are pathological differences between infestation due to S. haematobium from these two parts of Africa.

Proposed Course of the Project:

It is anticipated that these animals will be allowed to live with this chronic infection for as long as two or three years to see whether chronic bladder disease due to infestation with this organism will result in bladder cancer in the hamster.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 525
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,900	$3,150	$11,050	0	1	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	1	1	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None.

NO ENTRIES FOR 15, 16 or 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 527
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Morphology, Pathogenesis and Transplantability of Spontaneous Neoplasms within the Canine Species.
PROJECT TITLE

7. Dr. Louise S. Lombard
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: The objective of the project is to study the morphology and pathogenesis of spontaneously occurring neoplasms within the canine species and to obtain transplantable canine neoplasms which can be utilized in morphologic, chemotherapeutic, metabolic, endocrinologic and irradiation studies.

Methods Employed: Through the use of total body irradiation, a spontaneous anaplastic thyroid carcinoma is transplanted by various routes in heterologous puppies. Numerous spontaneous canine malignancies are screened in the same manner. All tumors are followed histologically.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Major Findings: The transplantable canine thyroid carcinoma grows well in irradiated heterologous puppies. The morphology of the tumor remains essentially unchanged throughout a long series of transplant generations. Some 15 malignant neoplasms of different types have been transplanted to irradiated heterologous puppies and have failed to grow.

Significance to Cancer Research: Transplantable malignant neoplasms in the dog would offer tumors in a larger host for morphologic, metabolic, biochemical, hormonal, surgical and irradiation studies, as well as including another species for the testing of chemotherapeutic substances.

Proposed Course of Project: Spontaneously occurring neoplasms will be transplanted within the canine species utilizing total body irradiation, hypophysectomy and hormone preparation. The relationship between total body irradiation and the transplantability of the canine thyroid carcinoma will be studied by biochemical, immunologic and endocrinologic means.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 527
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$15,[illegible]	[illegible]	$22,0[illegible]	1	1	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 527
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Lombard, L. S. Transplantability of a Canine Thyroid Carcinoma through thirty generations in mixed-breed puppies. J. Nat. Cancer Inst. 17: 123-129, 1956.

Lombard, L. S. Transplantation of a Spontaneous Canine Carcinoma to the central nervous system of puppies. J. of Neuropathology & Exp. Neurology. In press

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

President, Woman's Veterinary Medical Association.

Member - American Society of Experimental Pathologists
Member - American Association for Cancer Research
Member - Federation of American Scientists

Note: Collaborative research is being done with Dr. W. R. Bryan on the study of the histologic picture of the Rous Sarcoma in seven breeds of chickens and in turkeys; with Dr. H. P. Morris on pituitary and hepatic neoplasms resulting from the administration of various carcinogens to rats; with Dr. H. L. Ratcliffe, Philadelphia Zoological Garden, on the relative frequency of neoplasms in captive wild animals; with Dr. T. Reed, National Zoological Park, on the relative frequency of neoplasms in captive wild animals at the Washington, D.C. Zoological Garden.

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Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 528
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Cytology of Ascites Tumor Cells
PROJECT TITLE

7. Dr. Robert Love
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To study the cytology and cytochemistry of ascites tumor cells during multiplication and degeneration as a means of investigating the structure and function of the neoplastic cell under different conditions.

Methods Employed: (a) Application of standard histochemical procedures and the development of a new stain for the demonstration of ribonucleic acid in the parachromatin of the ascites tumor cell. (b) Study of the morphology and distribution of the parachromatin and the nucleolus in relation to cell division.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Major Findings: 1) The transfer of ribonucleic acid from the nucleus to the cytoplasm of the ascites tumor cell has been shown to occur in two ways. a) By extrusion of the nucleolar remnant into the cytoplasm at the end of prophase. b) By liberation of parachromatin bodies into the cytoplasm where they can be detected in metaphase, anaphase and telophase. 2) In preliminary studies, parachromatin bodies have been demonstrated in the cytoplasm of human carcinoma cells, but have not been seen in normal epithelium.

Significance to Cancer Research: This type of transfer of ribonucleic acid from nucleus to cytoplasm during mitosis may be a characteristic of the neoplastic cell or merely a feature of rapidly dividing cells.

Proposed Course of Project: The study of the ribonucleic acid of the nucleus and cytoplasm will be extended to other adult, embryonic and neoplastic cells and will involve the development of more sensitive staining procedures. The effect of ribonuclease and, later, chemotherapeutic agents on the transfer of RNA from nucleus to cytoplasm will be studied.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 528
SERIAL NUMBER

12. BUDGET DATA:

ESTIMATED OBLIGATIONS			MAN YEARS		
DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
$7,900	$3,150	$11,050	1/2	1/2	1

FY'57

BUDGETED POSITIONS			PATIENT DAYS
PROF	OTHER	TOTAL	
1/2	1/2	1	0

FY'57

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 528
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Love, R., Orsi, E. V., and Koprowski, H. Cytological and cytochemical properties of ten ascites tumors. J. Biophys. and Biochem. Cytology 2: 1-14, 1956.

2. Love, R. The nucleolus and parachromatin of the ascites tumor cell. J. Biophys. & Biochem. Cytology. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 529
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Viral Therapy of Tumors
PROJECT TITLE

7. Dr. Robert Love
PRINCIPAL INVESTIGATOR

8. Dr. R. C. Suskind, Dr. R. J. Huebner
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To study the effects of infection of tumors with viruses and to enhance the oncolytic properties of viruses which have little or no pathogenicity for man.

Methods Employed: a) Passage of a naturally occurring strain of Newcastle disease virus and a strain which is oncolytic for a mouse tumor, in tissue cultures of HeLa cells followed by passage in the same cells grown in cortisonized irradiated rats. b) Study of the cytological and histological changes following infection of HeLa tumors grown in cortisonized irradiated rats.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Major Findings: 1) Growth of the original and the mouse tumor adapted strains of Newcastle disease virus (NDV) has been established in HeLa cells in tissue culture and in the rat. On serial passage in tissue culture only the adapted strain is cytopathogenic. 2) Infection of HeLa tumors grown in the peritoneal cavity of irradiated rats with Dr. Suskind's Coxsackie B3 virus results in a characteristic type of cell degeneration which resembles that which has been observed in virus-induced oncolysis occurring in mouse and chicken tumors. 3) Histological evidence of regression of HeLa tumors grown in the peritoneal cavity has been observed after administration of the virus by the oral, intraperitoneal and intravenous routes.

Significance to Cancer Research: The adaptation of viruses to develop a specific cytopathogenic effect on human neoplastic cells seems a promising chemotherapeutic approach. To this end, a study of the effects of such viruses on different types of tumors in animals and man is essential.

Proposed Course of Project: 1) To adapt N.D.V. virus to become oncolytic for human tumors. 2) To study the effects of infection of human tumors with viruses which have developed oncolytic properties for neoplastic cells of human origin grown in laboratory animals. 3) To investigate the nature of virus-induced regression in HeLa tumors grown under different conditions in animals.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 529
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,900	$3,150	$11,050	1/2	1/2	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	1/2	1	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 529
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Suskind, R. G., Huebner, R. J., Rowe, W. P., and Love, R. Studies of viral agents oncolytic for human tumors in a heterologous host. The oncolytic effect of Coxsackie B viruses. Proc. Soc. Exp. Biol. & Med. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 530
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Acquired Tolerance.
PROJECT TITLE

7. Dr. Robert Love
PRINCIPAL INVESTIGATOR

8. Dr. H. Koprowski
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To apply to principle of acquired tolerance to grow HeLa cells in the mouse.

Methods Employed: Foetal mice are injected with HeLa cells in order to produce tolerance to a subsequent intracerebral injection of the same cells after birth.

Major Findings: Four HeLa tumors were found in sick mice which were killed 2 to 3 weeks after birth.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 530
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Koprowski, H., Theis, G., and Love, R. Tumor. Transplantation Bulletin 3: 55-56, 1956.

Koprowski, H., Theis, G., and Love, R. Immunological Tolerance in Tumour Studies. Adaptation of Ascites Tumours to Homologous or Heterologous Hosts. Proc. Roy. Soc., London, 146: 37-58, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 531
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Study of Filterable Agents Isolated from Mouse Tumors.
PROJECT TITLE

7. Dr. Robert Love
PRINCIPAL INVESTIGATOR

8. Dr. H. Koprowski
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To study the effects of inoculation of newborn and foetal mice with cell-free filtrates of Ehrlich ascites tumor.

Methods Employed: AL/N and AKR X C3Hf/He mice are inoculated as indicated above.

Major Findings: Inconclusive at this stage.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Significance to Cancer Research: Any new light on the so-called "viral etiology" of mouse tumors will be valuable.

Proposed Course of Project: To continue observation of inoculated mice.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 531
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,950	$1,560	$5,510	-	1/2	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	1/2	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16, or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 532
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Pathology of Poliomyelitis of the Chick Embryo (completion of previous wor
PROJECT TITLE

7. Dr. Robert Love
PRINCIPAL INVESTIGATOR

8. Dr. M. Roca-Garcia
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To study the pathogenesis of poliomyelitis in the chick embryo.

Methods Employed: Study of the histological changes and of the distribution of virus after inoculation of embryos by the yolk sac and intravenous routes.

Major Findings: Results suggest that virus enters the nervous system at many different points directly from the blood. Axonal transmission of the virus seems to play a small part in the evolution of the disease.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Significance to Cancer Research: None.

Proposed Course of Project: Completed.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 532
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,950	$1,560	$5,510	-	1/2	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	1/2	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 532
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Love, R., and Roca-Garcia, M. Mode of infection of the nervous system in poliomyelitis of the chick embryo. Nature 178: 746-747, 1956.

Love, R., and Roca-Garcia, M. Pathogenesis of poliomyelitis in the chick embryo. Archives of Path. In press.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 533
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Pathology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Induc. & Pathogenesis
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Induction of Thymomas in Mice by Transplantation of Thymus Tissue into Spleen.
PROJECT TITLE

7. Dr. Roger W. O'Gara
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: Studies performed at the University of Pennsylvania Hospital on thymomas and other anterior mediastinal tumors in man stimulated my interest in the etiology and development of thymomas. This work is currently being prepared for publication. The present project attempts to induce thymomas in mice by transplantation of thymic tissue into the spleen.

Methods Employed: Thymic tissue from newborn C57Bl mice was transplanted into the spleen of thymectomized 6 week old mice of the same strain. A corollary experiment using irradiated mice as the host animal is now being completed.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd)

Major Findings: Operative procedures are nearing completion, and it is anticipated that 6 months or more will be required before tumors appear.

Significance to Cancer Research: The study will discover whether thymomas can be produced by this method and if successful, will shed light on the pathogenesis and comparative morphology of these tumors.

Proposed Course of Project: (1) Study of development of any induced tumors by selected necropsy and/or biopsy.

(2) Study of possible endocrine relationship of the tumors by transplantation and other means.

(3) Study of possible relationship to myasthenia gravis.

(4) Comparison of thymomas with lymphomas induced in irradiated animals.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 533
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$19,740	$7,870	$27,610	2	1/2	2-1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2	1/2	2 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

NO ENTRIES FOR 15, 16, or 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-624
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Radiation Injury and Repair
PROJECT TITLE

7. H. L. Andrews
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: This is a change and extension of Serial No. 624 previously submitted. The objective is to gain insight into the mechanisms by which ionizing radiations affect living tissues.

(Use reverse and additional pages, if necessary)

10. Continued

Methods employed: It has been necessary to restrict the studies from those described in last years report. Survival times only have been studied, without the concomitant investigations originally planned.

Major findings: Survival times have been determined in mice, rats, hamsters, and guinea pigs at doses up to 100,000 r using x-rays from the 3 Mev Van de Graaff generator. Some work on dose fractionation has been done but this is not complete. The data are being treated mathematically in terms of a simplified model in the hope of obtaining a better understanding of the mechanisms involved.

Significance to cancer research: Any increase in knowledge of the biological effects of radiation is of potential value in improving radiation therapy.

Proposed course of project: Fractionation studies, which should shed considerable light on repair processes are not complete. These should be completed if possible. Other extensions depend to some extent on the results of the mathematical analysis.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-624
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-628
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Dosimetry of High Energy Radiations
PROJECT TITLE

7. H. L. Andrews
PRINCIPAL INVESTIGATOR

8. R. E. Murphy
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To develop methods and instruments for determining the radiation doses delivered to tissues at energies up to 3.5 Mev and at high dose rates.

Methods employed: The usual types of ionization chambers used for radiation dosimetry are not designed to read correctly at the x-ray

(Use reverse and additional pages, if necessary)

10. Continued

energies available from the 3.5 Mev generator and are probably incapable of measuring the high intensities anticipated when the generator is run at full output. Chemical dosimeters appear to have a response independent of photon energy, and to be capable of accurately recording high dose rates. Work has concentrated on two chemical systems, the production of HCl from chloral hydrate and the oxidation of ferrous iron to ferric.

Major findings: The gel dosimeter previously developed has been applied to electron dosimetry in connection with patient irradiations. Improved resolution has been obtained by determining dosage from an electrical conductance measurement instead of the pH method previously used.

Significance to cancer research: A most basic requirement for good radiation therapy is that the tumor dose be made as high as possible relative to the dose delivered to healthy tissue. Any method which can improve the measurements of dose delivered to deep body structures should improve the ability of the therapist to keep the tumor/tissue dose ratio high.

Proposed course of project: Measurements at high dose rates have not been made because of target failures when attempts were made to operate the generator at high power. As this difficulty is overcome chemical dosimetry will be applied to high dose rates and to short pulses of both x-rays and electrons. These measurements will then provide a basis for the extension of research in radiation biology into a dose-rate range never before reached in the laboratory.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-628
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):
None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-629
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The Effect of Ionizing Radiation on Amino Acids
PROJECT TITLE

7. Charles R. Maxwell
PRINCIPAL INVESTIGATOR

8. Dorothy C. Peterson
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The objective of this project is to determine the mechanism of the chemical reactions induced by ionizing radiation in aqueous solutions of amino acids. This is part of a long range program to accumulate information on simple systems of biological interest so that general principles may be ascertained and applied to complex systems which are not amenable to thorough, direct investigation.

Methods employed: Solutions of amino acids are irradiated with ionizing radiation and then analyzed for the products formed. Since the products

(Use reverse and additional pages, if necessary)

10. Continued

are sensitive to radiation it is necessary to determine the yield of each product as a function of dose to as low a dose as possible and to extrapolate these values to obtain the initial yield of the product at very low doses before secondary reactions distort the picture.

Once the products of the simple water-amino acid-radiation system are found the effect of the introduction of other chemicals into the system are studied. Substances are selected, in accordance with the radidly expanding concepts of radiation chemistry, which are thought to accentuate or repress one or more of the various intermediate processes by which radiation is believed to produce its effects.

Since the final chemical (and ultimate biological) effect is the result of the formation of a complex microscopically-heterogenous mixture of excited molecules, H_2O_2, H, and OH free radicals in discrete "tracks", the influence of the physical distribution of these active intermediates as well as possible differences in relative abundance and/or chemical nature is investigated by using different sources of radiation. To date 50 KV x-rays, 150 KV electrons, and 5 Mev alpha particles have been used.

One of the more important, if not the most important, active intermediates is the OH free radical. The role of this radical in the absence of the H free radical and the host of excited molecules in the ionization track is studied by chemically producing this radical in the system being studied. To date this has been accomplished by the action of Fe^{++} and H_2O_2.

Major findings: Earlier work has shown that x-rays induce four reactions in aqueous solutions of both glycine and alanine. One of these reactions was shown to result directly from the absorption of energy by the dissolved molecule; the other three were shown to be indirect, e.g., the result of energy absorbed by the water which is generally considered to produce H and OH free radicals and H_2O_2.

This years findings fall into two catagories: findings dealing directly with the effect of ionizing radiation on glycine and findings of a supporting closely allied nature pertinent to the understanding of a basic mechanism. In the latter catagory a mechanism for the action of chemically produced OH free radicals upon glycine has been firmly established and is in preparation for publication. This mechanism is in almost complete disagreement with the two mutually consistent general schemes accepted for the last 5 to 8 years for the action of OH radicals and Fenton's reagent on organic substrates. The mechanism is not inconsistent with data upon which the other two schemes are based but with the schemes themselves which were formulated in the absence of kinetic data of the type we are now able to procure. If, as experiments now in progress indicate, this mechanism is applicable to most organic substrates, this will be a major contribution to free radical chemistry which is ultimately the basis of radiation chemistry.

The analytical technique for simultaneously measuring the Fe^{++} and H_2O_2 concentrations and securing the kinetic data upon which the above mechanism is based was developed in this laboratory and will be the source of a separate publication.

10. Continued

More directly, a tentative scheme, which is being further tested, has been formulated for the mechanism of the action of radiation on glycine. This scheme is based on the results of irradiations in the presence of other substances such as H_2O_2, O_2, H_2, Fe^{++}, Fe^{+++} and the products of the simpler system and upon the mechanism just mentioned. The scheme has two major points of interest. First, it outlines the role of all of the generally accepted active intermediates and is an extension of a simpler scheme proposed by E. G. Hart for the simpler compound formic acid. As such, it is potentially the basis of a general scheme which may be used to understand more complex systems. The second point of interest which may be of even more significance is that this scheme describes the role of all the generally postulated intermediates and yet provides no method for the formation of one of the major products of the gross reaction. This suggests that in concentrated solutions, which we have used almost exclusively, there is an active intermediate which is of little importance in the very dilute solutions commonly used by other investigators with simpler substrates. Since the more concentrated solution more closely approximates a biological system than does the very dilute solutions, this is potentially of great biological significance.

Significance to cancer research: Although not directly connected with Cancer research, it is of great interest because it seeks an understanding of the mechanism for the effects of radiation which is used empirically as a tool in the clinical treatment and laboratory study of cancer.

Proposed course of project: Continuation of the work will be directed toward:

1) Testing the generalization of the scheme established for the action of OH radicals and Fenton's reagent on glycine.

2) Testing the scheme now tentatively proposed for the action of radiation on glycine.

3) Testing the scheme in 2 as to its applicability to other compounds.

4) Establishing the role for the formation of the compound (HCHO) which as yet does not fit into any scheme.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-629
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$21,030	$8,340	$29,370	2	2/3	2-2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2	2/3	2 2/3	0

13. BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-634
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Late Effects of Irradiation in Animals Protected from Early Death by Bone Marrow or Antibiotic Treatment
PROJECT TITLE

7. Willie W. Smith, pathological studies by George Brecher (Clinical Center), Katherine Snell, Richard Swarm
PRINCIPAL INVESTIGATOR

8. Ilo M. Alderman, Ruth Gillespie
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To determine the extent to which life expectancy is reduced in animals which have been exposed to severely damaging radiation and then treated in such a way as to prevent early death from infection, hemorrhage or anemia and to determine where possible, the cause of death in these animals.

(Use reverse and additional pages, if necessary)

10. Continued

Methods employed: Animals exposed to radiation which causes death in the absence of treatment are treated with bone marrow or streptomycin and are then observed for the remainder of their lives. At death autopsies are performed and histopathological studies made. Various treatments are applied with the object of prolonging life.

Major findings: Essentially the same as previously reported. Histopathological studies are not complete.

Significance to Cancer Research: (1) An evaluation of the total efficacy of treatments which may be used to reduce the deleterious effects of therapeutic treatment with x-ray; (2) an estimate of the incidence and type of neoplasm which may be expected after whole-body exposure to varying doses of radiation; (3) an estimate of the physiological condition of the animal after exposure to varying doses of radiation.

Proposed course of project: No further experimental work planned for the present.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-634
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,880	$3,140	$11,020	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):
None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. CRP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-636
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Lethal Effect of Visible Light on a Mutant Strain of Haploid Yeast. I. General dependencies.
PROJECT TITLE

7. Dr. Mortimer M. Elkind
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The objectives of this research have been to delineate the parameters which control the development of light sensitivity in a respiration deficient strain of haploid Saccharomyces cerevisiae.

Methods employed: Actively growing (log phase) yeast cells were harvested from a liquid growth medium (yeast extract plus dextrose), washed by

(Use reverse and additional pages, if necessary)

10. Continued

centrifugation, and resuspended in a potassium phosphate buffer. Immediately after resuspension, the cells were essentially insensitive to the emission from a 300 watt, incandescent lamp slide projector (350 m μ to 750 m μ). With time, the population became increasingly light sensitive to this emission as measured by the ability of the cells to grow into visible colonies when plated on agar containing growth medium. The cells were irradiated in the same buffered solution as they were stored in, and were kept at a temperature of from 1.0-2.0° C during irradiation.

Major findings: A. Through experiments employing different concentrations of cells and filtrates of high titer suspensions, it was shown that the primary light absorption event is in the cell and is not abetted by the presence of dissimilation products in the storage buffer. B. For a given composition of the buffer and a given temperature of storage, with time the cells became progressively more light sensitive. C. For the same length of time of storage the sensitivity was strongly dependent on the temperature of storage. For instance, 2 hrs. of storage at 30° C produced the same sensitivity as about 28 hrs. storage at 6.9° C. D. For the same length of time and temperature of storage, the sensitivity was strongly dependent upon the pH of the buffer solution and to a lesser extent upon its molarity. E. Suppression of oxygen tension of the buffer solution at the time of irradiation decreased the sensitivity. F. Compared to log phase cells, resting cells or log phase cells of the parent diploid strain did not develop appreciable light sensitivity in the course of storage. G. Compared to other types of radiation, exposure to visible light markedly reduced the fermentation rate of light sensitive cells. At pH 7.0 and 30° C, no appreciable oxygen consumption was detected with dextrose using manometric techniques.

The preceding plus evidence from other work suggests that the site(s) of the photo-lethal effect is probably extranuclear and that it may be associated with the cell wall. The strong oxygen requirement implicates photodynamic action even though an absorbing pigment was not added to the system.

Significance to cancer research: This research has been a study of an effect not reported previously in yeast. It will aid in the understanding of the biological effects of radiation in general. Although the latter in itself is of use in cancer research, the main significance of this research lies in its documentation of a new biological phenomenon.

Proposed course of project: This work has been completed and is being submitted for publication.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-636
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$9,210	$3,650	$12,860	1/2	2/3	1-1/6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	2/3	1 1/6	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):
None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-637
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Development of High-Intensity X-ray Source
PROJECT TITLE

7. H. L. Andrews
PRINCIPAL INVESTIGATOR

8. R. E. Murphy
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To obtain from the 3 Mev generator the x-ray output which it should be capable of producing.

Methods employed: The 3 Mev Van de Graaff generator given to NIH by the Liggett and Meyers Tobacco Co. is capable of producing x-rays at intensities never before reached in the laboratory. It had never been used as an x-ray generator and when attempts were made to utilize its

(Use reverse and additional pages, if necessary)

10. Continued

capabilities x-ray targets failed by melting. Failures occurred at only about 1/4 of full power and hence the possible generator capability is seriously restricted. Since target failure allows cooling water to enter the accelerating tube each failure represents a minor disaster. Pending remedial steps the generator has been operated conservatively to insure continuity of service. Improved operation is to be expected from: 1. higher voltage operation, 2. high frequency target scanning, 3. use of targets of high atomic number, 4. improved target cooling.

Major findings: No positive findings were obtained during the year in spite of considerable effort. The high speed scanning proved to be impractical since the high frequency magnetic fields required would not penetrate undistorted through the metal tubing containing the electron beam. Eddy current losses in either aluminum or stainless steel tubing proved to be excessive for any practical power source.

Two thorium targets were constructed and both failed, the second with a disastrous filling of the acceleration tube with cooling water. Since failure occurred with a power input to the target of only about 3 kilowatts the thorium represents no improvement over the conventional gold. If a satisfactory thorium-stainless steel weld could be developed the thorium target might be useful for higher powers.

Significance to cancer research: With the high outputs potentially available from this generator radiation doses can be administered at dose rates never before available. It is possible that quite different biological results will be obtained when a given radiation dose is given at say 10,000 r/min rather than at the more usual 50-100 r/min.

Proposed course of project: It is now felt that a liquid mercury target may be capable of handling large amounts of power. Preliminary steps have been taken to construct a suitable mercury target.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-637
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,880	$3,140	$11,020	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):
None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-639
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. A Study of the Life Span of the Nucleated Red Cell
PROJECT TITLE

7. Kirkland C. Brace, M. D.
PRINCIPAL INVESTIGATOR

8. Paul D. Altland
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: A better understanding of the remarkable resistance of the lower animals and particularly the poikilotherms, to the effects of ionizing radiation.

Methods employed: The erythrocytes of various species of animals are labeled in vivo by the intravenous or intraperitoneal injection of glycine -2-C^{14}. The methyl carbon is incorporated into the cell

(Use reverse and additional pages, if necessary)

10. Continued

protein and retained until the cell is destroyed. The determination of the life span is based upon the interpretation of the change of the activity of the cell protein following injection of the glycine.

Major findings: Recently published data on the duck and the chicken indicate that these nucleated cells are relatively short lived. Our value of 20 days for the chicken red cells is somewhat shorter than previously reported values. Our figure of 39 days for the duck is substantially greater than that reported by other investigators. The work on the turtle and the amphibious toad is still incomplete. The activity of the cell protein of the toad red cells is essentially unchanged after 18 months and the turtle cells still retain their activity after 24 months. This can only be interpreted as 100 per cent reutilization of the labeled material or that the cells are extremely long lived.

Significance to Cancer research: The peculiar physiology of the red cells of the poikilotherms is probably related to the remarkable resistance of these species to radiation injury.

Proposed course of project: The studies on the birds have been terminated. The studies on the amphibians and reptiles are set up to continue for another year in an effort to determine if a finite life span can be obtained.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-63 [redacted]
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

National Institute of Arthritis and Metabolic Diseases

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI-639
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Kirkland C. Brace and Paul D. Altland, Life Span of the Duck and Chicken Erythrocyte as Determined with C^{14}. Proceedings of the Society for Experimental Biology and Medicine 92:615, 1956

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-640
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Effect of 100% Oxygen at Atmospheric Pressure on Antibody Production
PROJECT TITLE

7. Falconer Smith
PRINCIPAL INVESTIGATOR

8. Marie M. Grenan and H. Jeanette Ruth
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine the extent of the parallelism between the oxygen-effect and the radiation-effect on antibody synthesis.

Methods employed: Exposure to high partial pressures of oxygen are carried out at various time sequences with respect to the time of immunization with sheep erythrocytes. Peak hemolysin titers are compared in mice exposed to high partial pressure of oxygen.

(Use reverse and additional pages, if necessary)

10. Continued

Major findings: So far as is presently known the inhibitory effect on antibody production resulting from exposure to high oxygen tensions after immunization as a new finding may be of considerable importance.

Significance to cancer research: This finding, if general and well substantiated, would present a useful means of preventing antibody synthesis in place of radiation which has unfavorable collateral effects related to hematopoiesis.

Proposed course of project: It is expected that additional studies will have to be carried out using pressure controls. Further work at higher than atmospheric pressure is indicated. The importance of the time relationships of immunization and antibody induction phase will be studied.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-640
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$9,210	$3,650	$12,860	1/2	2/3	1-1/6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	2/3	1 1/6	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-641
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The Effect of Irradiation on Susceptibility to Viral Infections
PROJECT TITLE

7. Willie W. Smith, Bernice Eddy (L.B.C.)
PRINCIPAL INVESTIGATOR

8. Ilo M. Alderman, Ruth Gillespie
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine whether or not exposure to x-radiation alters susceptibility to various viral infections.

Methods employed: The response to challenge with poliomyelitis or influenza virus in mice given just sublethal irradiation is compared with that of controls.

(Use reverse and additional pages, if necessary)

10. Continued

Major findings: The susceptibility of mice to challenge with polio virus was not significantly increased by just sublethal irradiation. Passage of a non-adapted mouse strain of influenza virus through sublethally irradiated mice increased it virulence. In general, the differences in susceptibility of irradiated and control mice to the viruses tested were not pronounced.

Significance to Cancer Research: Since generalized radiation damage may be encountered as a side effect of cancer therapy it is obviously important to understand alterations in susceptibility to infection, both bacterial and viral. Results of these experiments are also of interest in connection with the use of viruses as therapeutic agents.

Proposed course of project: This project is near completion and we anticipate no major expansion at present.

Form No. CRP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-641
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):
None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-642
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Antibody Production in Irradiated Mice: Effect of Chronic, and Intermittant Exposure to Ionizing Radiation and Fractional Body Shielding on Hemolysin Production.
PROJECT TITLE

7. Falconer Smith
PRINCIPAL INVESTIGATOR

8. H. Jeanette Ruth and Marie M. Grenan
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To determine from the immune response of the mice given the indicated treatments the nature of the injury inflicted on the mechanism of antibody synthesis by ionizing radiation.

Methods employed: Peak serum antibody titers are determined by chemical means following both repeated and chronic exposures to predetermined amounts of ionizing radiation. Fractional body shielding is carried out by lead grids exposing varying volumes of the body.

(Use reverse and additional pages, if necessary)

10. Continued

Major findings: A total accumulated dose of 450 r to the whole body over a period of 51 days has been found to result in a significantly lowered average peak hemolysin titer compared to mice of equal age and weaning lot.

Average hemolysin titers of mice exposed to divided doses of varying amounts but equal to 450 r total show that the degree of inhibition is directly related to elapsed time between mid dose and immunization. The results show also that there is a cumulative non-recoverable damage at least within the 14 day period studied, resulting from 20 daily doses of 22.5 r x-rays.

Peak titers are only slightly depressed in mice immunized at 7 days and not significantly lowered at 14 days in mice exposed to 1140 r (LD_{75}) to 66% of the body weight in these mice, hematopoietic activity is markedly lowered.

Significance to cancer research: Elucidation of the damage inflicted by ionizing radiation to protein synthesis and cellular metabolism is inherent in the problems of the therapeutic use of radiation.

Proposed course of the project: Fractional body shielding is essentially complete while experiments on the effects of chronic radiation exposures will continue using higher total doses and varied divided doses.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-642
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$9,210	$3,650	$12,860	1/2	2/3	1-1/6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	2/3	1 1/6	0

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

October 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards and Publications

15. NCI-642
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Antibody Production in Mice Fractionally Shielded During Exposure to X-Rays - Falconer Smith, H. Jeanette Ruth, and Marie M. Crenan

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-643
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Long Term Survival and Tumor Incidence Following Acute or Chronic Irradiation
PROJECT TITLE

7. Joanne Hollcroft
PRINCIPAL INVESTIGATOR

8. Eliza Miller, Charles C. Congdon
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

A. To study long term survival and carcinogenesis of:

1) Chronic irradiation of guinea pigs followed by injections of bone marrow.

2) Acute radiation doses when the animals are protected from the acute irradiation lethality.

(Use reverse and additional pages, if necessary)

10. Continued

B. Methods employed:

1) Strain 2 guinea pigs were exposed to 8.8 r per day gamma irradiation. When their hematocrit number dropped to about 25 they were removed from the radiation field. At this time half the pigs were given intravenous injections of bone marrow. Survival and tumor incidence are being studied. Survival and tumor incidence are also being studied on strain 2 guinea pigs exposed to 4.4 r per day and removed from the irradiation field when their hematocrits dropped to 30.

2) C_3H_f mice were x-irradiated under the following conditions:

(a) 400 r at birth
(b) 400 r with sham spleen shielding
(c) 400 r with spleen shielding
(d) 900 r with spleen shielding, and
(e) 900 r with chemical protection

C. Major findings:

1) Intravenous injections of homologous bone marrow decreased the number of early deaths following limited chronic irradiation. The females appear to be more sensitive to the irradiation. Leukemia is not prevented in the females by bone marrow suspensions. Of the animals in which the blood picture recovered following removal from the field, the bone marrow treated animals seem to have a longer survival time. Leukemia is also occurring in guinea pigs exposed to the lower dose rate of 4.4 r per 8 hour day.

2) Studies on survival of C_3H_f mice following single doses of x-irradiation showed that:

(a) Control females lived longer than control males, but no sex difference in survival time was noted in mice irradiated at birth or in those irradiated while undergoing spleen or sham spleen shielding. Male mice protected with anoxia and cysteine against 900 r lived longer than females similarly treated.

(b) Mice irradiated at maturity lived longer than mice irradiated at birth.

(c) Long term survival time was not influenced by spleen protection but was dependent on dose.

(d) Cysteine and anoxia protection produced a dose reduction effect reflected by a life span longer than that seen in spleen protected mice receiving equivalent radiation treatment.

(e) A given dose of radiation was more carcinogenic to the females than to the males. The number of adrenal and ovarian tumors was consistently increased. Carcinogenicity was influenced by spleen protection only in that lymphosarcoma induction was decreased. Tumor types not seen in control animals were seen in irradiated mice.

(f) Glomerulosclerosis was prevalent in mice receiving ordinarily lethal doses while pyelonephritis in males was reduced by all irradiation treatments.

10. Continued

Significance to Cancer Research: Irradiation is a well known carcinogen. The long-term effects of acute doses of total body irradiation are of consequence in considering the role of radiotherapy in treatment of cancer.

Proposed course of project: 1) Influence of hematocrit number and white blood count as well as total dose on development of leukemia and modification by therapy during the irradiation.

2) This project is completed and no further studies along this line are proposed at present.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-643
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,880	$3,140	$11,020	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Dr. C. C. Congdon, Oak Ridge National Laboratory

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-644
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Modification of X-irradiation Treatment of Localized Tumors.
PROJECT TITLE

7. Joanne Hollcroft
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine in what manner the tumor reacts to ionizing radiation and in what manner the radiosensitivity may be increased.

Methods employed: Study the radiosensitivity of transplanted tumors under various conditions. Lymphosarcoma, L-1, a fibrosarcoma and a plasma cell sarcoma are being studied.

Major findings: a) The plasma cell sarcoma is relatively sensitive to radiation; the dose necessary to cure 50% of the tumors is about

(Use reverse and additional pages, if necessary)

10. Continued

2000 r. The sensitivity appears to change with time after transplant. The fibrosarcoma does not respond to doses below 5000 r. 5000 r to 10,000 r produces about the same number of cures.

b) Small total-body doses increase the radiosensitivity of both the plasma cell and the fibrosarcoma.

c) Radiosensitivity of L-1 has not changed after 10 generations when each generation is irradiated with 1000 r.

Significance to cancer research: To find methods of increasing usefulness of radiotherapy.

Proposed course of project: Comparison of fractionation treatment of radiosensitive tumors with that of radio-resistant tumors. Further studies on radiation induced radio-resistance. Use of antimetabolites and cytotoxic poisons to potentiate radiation effects.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-644
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,880	$3,140	$11,020	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. CRP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-645
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Effect of X-irradiation on Disseminated Lymphosarcomas, L-2, L_2C.
PROJECT TITLE

7. Joanne Hollcroft
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine the LD_{100} for L-2 tumor and devise a feasible treatment schedule.

Methods employed: Subcutaneous and ascitic transplants of L-2 tumor are used for treatment. The bioassay technique is used to ascertain effectiveness of radiation treatment.

In addition, studies are being done on short interval fractionation of total-body irradiation in an attempt to raise the lethal dose.

10. Continued

Major findings: More nucleated cells will live when transplanted immediately after irradiation than when transplanted 1 day following irradiation. The number of cells surviving transplant may increase again in the second day after irradiation.

No transplants of a single lobe of liver or whole spleen grew following 3000 r; no transplants of whole tumor grew following 4000 r.

Increased survival of non-tumor bearing animals is obtained by dividing a total body dose in equal parts spaced 4 hours apart.

Significance to cancer research: Radiotherapy of leukemia.

Proposed course of project: Use of the bioassay technique to study radiosensitivity of other disseminated neoplasms. Setting up of better treatment schedules.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-645
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-650(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Service Radiation Therapy
PROJECT TITLE

7. J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. Robert W. Swain
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To provide radiation therapy for those patients requiring such in the course of the disease for which they are being investigated by research groups other than the Radiation Branch and to study in the Radiation Branch the applicability of radiation therapy to cases which present either unusual technological problems, rare or unusual neoplasms, or unusual manifestations of cancer.

(Use reverse and additional pages, if necessary)

10. Continued

Methods employed: The methods of radiation therapy employed are about as variable as the case material which is included in this project. A wide range of photon energies is used and various methods of application are employed.

Patient material: The total patient material of the Radiation Branch consisted of 103 cases given 1900 radiation treatments in Calendar Year 1956. Represented in this material is a very wide variety of neoplasms in all the diagnostic categories of cancer including cancer of the central nervous system.

Major findings: Responses to radiation therapy have been consistent with those reported by others. In some instances, notably wide spread intra-abdominal cancer, dose and time relationships which have not been reported by others have been employed and some unusual and very useful palliative results have been secured.

Proposed course of project: To continue.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-650(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$10,590	$37,860	$48,450	1/3	2/3	1 1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	13 Patients, 440 Days
FY'57	2/3	2/3	1 1/3	

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-651(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Electron Beam Radiation Therapy Study of Mycosis Fungoides
PROJECT TITLE

7. J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. Eugene J. Van Scott, M. D., Howard L. Andrews, Robert W. Swain, Philip Rubin, M. D., Raymond Murphy, Richard Reinertson, M. D.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To study the symptomatic, histologic and other objective responses of patients with mycosis fungoides to radiation of the entire skin with high energy electrons.

Methods employed: In the calendar year 1956 the 3 mev Van de Graaff FD electron accelerator has been modified so that a clinically useful fan-shaped beam of electrons with an initial energy of 2.1 mev can be

(Use reverse and additional pages, if necessary)

10. Continued

removed in an appropriate fashion for experimental human cancer therapy. The maximum depth of penetration of this beam in tissue is 1.0 cm. Patients are moved under this beam on a mobile table and by rotating the patient into several different positions the entire skin surface can be radiated with the electron beam. Weekly radiations of approximately 300 rep to 450 rep have been given to the skin of the entire body at a single sitting with total doses of 1000 to 3000 rep. Continuous clinical observations are made and photographic and biopsy material has been obtained where appropriate.

Patient material:

Seven patients with mycosis fungoides have been studied systematically in respect to their responses to the experimental electron beam radiation therapy.

Major findings: Prolonged clinical improvement has occurred in six of the seven patients radiated. The longest period of time a patient has now been followed is 20 months. Partial and, in some areas, complete disappearance of lymphomatous infiltrates has consistently been observed, but in no case has complete eradication of the disease occurred on either clinical or histological bases. Histological studies have also shown degenerative changes occurring in the epidermis, pilosebaceous structures, sweat glands, dermal blood vessels, and collagen. The magnitude of these changes has been proportional to the dosage. No systemic effect attributable to radiation has been observed and this confirms what was predicted for all the radiation is absorbed within the first centimeter of tissue and where bone and bone marrow are superficially located, as over the thorax, absorption would be virtually complete in cortical bone. In other words, the method employed provides a means of intensive radiation of cutaneous and subcutaneous structures without radiation of sensitive bone marrow.

Proposed course of project: It is proposed to continue the project for refinements and technic are continuously being made and it is anticipated that such refinements may continue. The dose-response relationship is not yet clearly understood and a considerable amount of work remains to be done in this area. The limits of tissue tolerance have also not been clearly established and when such information has become available it will then be possible to determine whether or not in favorable cases the infiltrative tumor may be completely eradicated.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-651(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$9,440	$19,440	$28,880	[redacted]	2/3	11/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	10 Patients, 226 Days
FY'57	2/3	2/3	1 1/3	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-653(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The Influence of Oxygen in Modifying Radiation Response
PROJECT TITLE

7. J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. Philip Rubin, M. D., and Robert W. Swain
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To investigate the possibility of modifying response of neoplastic cells to ionizing radiation by altering the oxygen content of blood.

Methods employed: Appropriate patient material (see below) is irradiated over bilaterally more or less symmetrically enlarged lymph nodes with equal doses of ionizing radiation (250 KV x-rays) under different conditions of oxygen saturation of hemoglobin and dissolved

(Use reverse and additional pages, if necessary)

10. Continued

blood oxygen. One bilaterally symmetrical area is irradiated under conditions of normal atmospheric pressure and oxygen tension. The other bilaterally symmetrical area is irradiated under conditions of normal atmospheric pressure with 100% inspired oxygen under which conditions the differential oxygen tension is increased by a factor of approximately 5.

If a differential effect is observed it is proposed to extend the experiment by irradiation of similar patient material under conditions of reduced oxygen tension (normal atmospheric pressure but reduced differential oxygen tension) and under conditions of increased oxygen tension to above normal atmospheric pressure by radiation in a pressure chamber. It is further proposed to investigate directly the oxygen tension in the tissues exposed to ionizing radiations by the use of appropriate instruments.

Patient material: Cases of Hodgkin's disease of lymphosarcoma with bilaterally and symmetrically enlarged lymph nodes in previously untreated areas.

Major findings: Project was inactive in 1956 because of lack of appropriate patient material.

Proposed course of project: As indicated in methods above.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-653(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-654(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Time as a Modifier of Clinical Radiation Response
PROJECT TITLE

7. J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. Philip Rubin, M. D. and Robert W. Swain
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To evaluate the prolongation of treatment time as a modifier of clinical response to ionizing radiations.

Methods employed: Irradiation of appropriate patient material (see below) to tumor doses conforming with the equation,

$$D = 2750\ t^{0.23}$$

over approximately a 100 day treatment period. This amounts to a tumor dose of approximately 10,000 roentgens.

(Use reverse and additional pages, if necessary)

10. Continued

Patient material: This project has been pursued actively in calendar year 1956 and virtually all patients with cancer of the oral and pharyngeal cavities which have been seen by the Radiation Branch have been admitted to this project. In addition, some patients with cancer of the esophagus and with recurrent and metastatic cancer in the pelvis from carcinoma of the uterine cervix have also been studied under this program.

Major findings: Major findings can only be reported in brief at this time because the series is now under intensive study with a view to re-evaluating the project and reporting the results in the next six months. The project was undertaken because theoretical considerations suggested that the dose and time employed might be cancerocidal for squamous cell carcinoma but that, because of the prolonged time, damaging effects to normal tissues might be minimized. Failure of response of squamous cell carcinoma to doses of ionizing radiation which are damaging to normal and supporting tissues is a limiting factor in contemporary radiation therapy. Experience on the project has shown that the dose employed is not always cancerocidal and that squamous cell carcinoma may be persistent immediately following the completion of this dose. In some instances, however, there has been complete clinical disappearance of squamous cell carcinoma. At the same time effects on normal tissues have been severe. It was anticipated from previous experience with relatively short term radiation programs that mucosal reactions could be expected to appear and disappear despite the fact that radiation was continued. This has not turned out to be the case as in several instances persistence of cyclical recurrence of severe mucous membrane reactions occurred. Such reactions have resulted in severe debility in several patients. In addition, one case of extreme, relatively acute edema of the soft tissues of the neck has been observed. In two instances of pelvic radiation to this high dose level perforation of the small intestines has occurred and this could be related, on histological bases, to radiation damage.

All of the patients submitted to this project have been in a very advanced stage of their disease. This has modified the prognosis substantially and has necessitated the radiation of large volumes of tissues. Inasmuch as patient and normal tissue tolerance is also related to volume of tissue radiated it is not unexpected that extreme reactions might occur in these patients. Several unexpected and useful results have occurred and this especially relates to one or two instances of esophageal carcinoma. Whether this has been simply fortuitous or is related to the technic employed is impossible to determine at the present time. Despite the severe reaction described it has been the impression of the investigators that the general and local tolerance to the radiation technic employed has been better than what might be described as standard technic where a somewhat lower dose is given in a much shorter period of time. The most strikingly beneficial results have been observed where the tumor and, therefore, the total tissue radiated have been of limited extent. It is presumed, therefore, that the undesirable responses which have been observed are due as much to the volume effect as to the high dose employed and consideration is being given to this aspect.

10. Continued

Proposed course of project: To review and report experience to date and to modify the project as may be indicated or necessitated by the preliminary results.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-654(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$15,000	$105,270	$120,270	1/3	2/3	1 1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	30 Patients, 1223 Days
FY'57	1 2/3	2/3	2 1/3	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-655(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Determination of Radiation Tolerance to the Brain by means of Electroencephalographic Studies
PROJECT TITLE

7. Philip Rubin, M. D., and J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. Dr. Cosimo Ajmone Marsan
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The use of electroencephalographic studies while treating various brain tumors will be employed to determine if any damage has been done to normal brain tissue. It is assumed that physiological changes reflected in the electroencephalogram may precede the severe structural anatomical changes. No attempt has been made to correlate electroencephalographic changes in definite areas of the brain with isodose curves which will give exact dosage to these points.

(Use reverse and additional pages, if necessary)

10. Continued

Methods employed: Serial electroencephalographic studies will be done during the course of treatment, beginning with a base line study. Aberrations in brain waves in certain areas will be correlated with the dosage level at these points. With the sharp beam alignment and available isodose curves this can be calculated with sufficient accuracy to have considerable correlative values.

Patient material: Appropriate case material which includes a wide range of brain tumors which have proved to be inoperable, but show some evidence of radioresponsiveness on the basis of the literature.

Major findings: Major findings cannot be reported at this time because this project was initiated only within the past year.

Proposed course of project: It is proposed that in the next year this study will be considerably amplified in conjunction with an expanded activity in brain tumor research in the NINDB.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-655(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-656(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Attempt at Radiotherapy with S^{35}-Sulfate
PROJECT TITLE

7. Philip Rubin, M. D., and J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. Joanne Hollcroft, Marion Matthews, Raymond Gottshalk, M. D.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: S^{35} given as sulfate selectively concentrates in cartilage and tissues containing abundant sulfated mucopolysaccharides. Since the beta radiation of S^{35} is very weak (in the neighborhood of 50 kv.) it must be determined first whether this can destroy cartilage cells. The S^{35} is laid down in the ground substance of cartilage, a chondroitin sulfuric acid polysaccharide. It is postulated that the path of the emitted electrons will be adequate to reach the cells. To date, no

(Use reverse and additional pages, if necessary)

10. Continued

evidence of cell destruction has been shown with the dosage used in adult cartilages. It seems essential before one embarks on the use of large amounts of this radioisotope which will mean considerable expense, that evidence of some radiotoxic effect should be shown on cartilage.

Methods employed: Two experiments using litters of suckling and weaned rats were executed to determine the following points:

A. The dosage range that would effect cartilage growth without destroying the animal.

B. A fractionated injection schedule versus a single injection to produce maximal cartilage changes.

C. Ascertain the toxic effects and find that they could be lessened by different methods of injections described in B.

Patient material: Application to Patients with Chondrosarcoma: The above work suggests that growing cartilage can be destroyed by radiosulfur and an attempt will be made to utilize this agent on a clinical basis. Only patients with advanced chondrosarcoma who have been treated by conventional methods will be considered candidates for this type of treatment.

Major findings: Conclusions of Experiments: (1) Minimal dose to arrest cartilage growth is 0.1 millicurie per gram of body weight. The changes are severe and result in an irreversible destruction and alteration of cartilage in the metaphyses and epiphyses. Reversible changes are noted with the lower doses employed. (2) When this dose, 0.1 millicurie per gram administered three times biweekly intervals to the 0.3 millicurie per gram level it would result in more profound cartilage growth changes than the 0.3 millicurie per gram given in the single injection. (3) The maximum tolerating levels are in the neighborhood of 0.3 millicuries per gram as a single injection since this has an LD25-30 with it. (4) A fractionated dosage schedule at the 0.3 millicurie per gram level is more effective in arresting and altering cartilage growth and less toxic symptomatically than a single injection at the same level. This has been correlated with tissue assay and radiomicrograph tissue sections.

Significance to Cancer research: A new method of treating chondrosarcoma.

Proposed course of project: Projection to patient level.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-656(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,880	$3,140	$11,020	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-657
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The Effect of Whole and Partial Body Irradiation on Bone Growth
PROJECT TITLE

7. Kirkland C. Brace, M. D.
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine the effect small doses of whole body x-irradiation and large doses of partial body irradiation upon the growth of the long bones of weanling rats.

Methods employed: The legs and whole body of the weanling rats are exposed to various doses of x-irradiation and the effect on body weight and the growth of the long bones is measured.

(Use reverse and additional pages, if necessary)

10. Continued

Major findings: Doses of x-rays as low as 1200 r delivered over the long bone of the leg produced a severe shortening of the bone which is permanent. The systemic effect of exposing such small areas as the leg is apparent in shortening on the unirradiated long bones and the production of dwarfs. It now appears that small partial body exposure to a large dose of x-rays is capable of affecting the growth of the entire animal.

Significance to Cancer research: These findings indicate some of the dangers of radiation therapy in growing children. Apparently even the irradiation of small areas produces an effect on overall body growth.

Proposed course of project: We propose to evaluate the partial body gram rad dose as compared to a similar whole body dose and the possible additive effect of the whole and partial body exposure on the growing bones.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-657
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1 2/3	2	0

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-658
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5. Johnson Research Foundation for Medical Physics, University of Pennsylvania, Philadelphia, Pa. and NCI, Bethesda, Md.
LOCATION (IF OTHER THAN BETHESDA)

6. Lethal Effect of Visible Light on a Mutant Strain of Haploid Yeast. II. Absorption and Action Spectra.
PROJECT TITLE

7. Dr. Mortimer M. Elkind
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: The objectives of this research were to: 1) determine the cytochrome deficiencies responsible for the respiration deficiency of the haploid Saccharomyces cerevisiae under study; 2) determine the absorption spectrum of any pigment the development of which with time correlated with the development of light sensitivity with time; 3) determine the action spectrum of the visible light lethal effect; and 4) correlate the results of 2) and 3) in an attempt to identify the hemochromogen responsible for the absorption event leading to lethality.

(Use reverse and additional pages, if necessary)

10. Continued

Methods employed: A. Spectroscopy - Absolute absorption spectra at liquid air temperature and difference spectra at 1° C were recorded at the Johnson Research Foundation using the exceptional equipment developed by Dr. Britton Chance and collaborators. This equipment possesses the sensitivity, resolution, and atuomatic recording requirements necessary to rapidly obtain spectra of dense suspensions of whole cells in the Soret and visible regions. Spectroscopy on intact cells precludes the possible introduction of artifacts which often can be produced in making cell homogenates or extracts. The use of suspensions of whole cells insured also that the conditions for the development of any new pigmentation were essentially the same as those required for the development of the photo-lethal effect.

B. Action Spectrum - The action spectrum was measured at the National Cancer Institute through the use primarily of gelatin cut-off filters. Though simple in concept and inexpensive to instrument, the results obtained in this way were crude compared to those obtainable with monochromator techniques.

C. Pigment identity - To aid in the possible identification of the hemochromogen detected in A and B, a series of experiments were performed at the National Cancer Institute using the irradiation techniques described in Project Report NCI-636. Various reagents and gaseous atmospheres were used to see if their effect could help identify the photo-sensitizing pigment.

Major findings: A. Respiration deficiency - From absolute and difference spectra it was found that the strain of cells under study lacked cytochromes a_3, a, c_1, and b. However, the cells do possess a complete respiratory chain from cytochrome c peroxidase through coenzyme I.

B. Photo-sensitizing pigment - From difference spectra, it was found that a hemochromogen develops linearly with time which has an absorption spectra characteristic of hemoproteins. The general shape of this spectrum and the location of its peaks agrees closely with the spectra of two known enzymes which resemble each other. These are the third complexes of H_2O_2 with catalase or peroxidase. Although not linearly related, the amount of this hemochromogen increases as the cells develop light sensitivity.

C. Action Spectrum - The integral curve for the sensitivity remaining with the use of a series of cut-off filters is consistent with the pigment described in B being responsible for the lethal action.

D. Pigment identity - Experiments performed with various modifying reagents and atmospheres do not entirely confirm the identification of the photo-sensitizing pigment as a complex III of catalase or peroxidase. Other pigments having similar absorption spectra are unknown to this investigator. This lack of a positive identification may be reasonable in view of the facts that complex III's have not been heretofore demonstrated *in vivo*, and hence their *in vitro* properties were used in interpreting the experiments referred to.

10. Continued

Significance to cancer research: That a new hemochromogen (probably a hemoprotein) has been demonstrated in a viable system in which it is associated with a photo-lethal effect is a new observation. The detailed understanding of such observations can be of significance to cancer research through the additional insight that they afford to biological systems in general.

Proposed course of project: The research described has been completed and is being submitted for publication. As time and conditions permit, it would be desirable to pursue this problem further by: 1) measuring the action spectrum with a high intensity monochromator; and 2) studying the processes subsequent to absorption which lead to lethality.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-658
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$9,210	$3,650	$12,860	1/2	2/3	1 1/6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	2/3	1 1/6	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-659
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Action of Endotoxin in Irradiated Animals: Reduced Mortality; Increased Resistance to Infection; Advanced Hematopoietic Recovery
PROJECT TITLE

7. Willie W. Smith
PRINCIPAL INVESTIGATOR

8. Ilo M. Alderman and Ruth Gillespie
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To determine the effects of bacterial endotoxins on the irradiated animal and to discover the physiological changes responsible for such effects.

Methods employed: Endotoxins are injected before or after irradiation and effects noted on: (a) mortality; (b) resistance to experimental infection; (c) hematopoietic recovery.

(Use reverse and additional pages, if necessary)

10. Continued

Major findings: (a) Reduction in mortality of approximately 20% when given after irradiation, 40% when given a day before irradiation; (b) Increased resistance to infection paralleling ability to mobilize granulocytes; (c) Advanced recovery in platelets, hemoglobin, and leucocytes and in cellularity of marrow and spleen.

Significance to cancer research: The advance in recovery of bone marrow cellularity following endotoxin treatment is of basic importance in cancer research. Induction of recovery in the irradiated marrow has hitherto been shown only following the injection of living cells, as nearly as one can judge from the literature. The importance of this observation is not limited to possible applicability of treatment, but extends to the general area of cell differentiation.

Proposed course of project: At present every effort will be made to demonstrate the presence of a substance in the serum of treated animals capable of stimulating recovery in irradiated marrow.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6[illegible]
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,880	$3,140	$11,020	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):
None

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. CRP-1 Calendar Year 1958
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-660(c)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Clinical Radiation Dosimetry
PROJECT TITLE

7. Robert W. Swain
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To develop methods for and to determine radiation dosage distributions for various types of radiation therapy used in the treatment of patients.

Methods employed: Chemical and thin-walled ionization chambers for electron beam therapy. Ionization chambers and photographic methods for Supervoltage X-ray Therapy.

(Use reverse and additional pages, if necessary)

10. Continued

Major findings: The average dose has been determined for the electron beam using ferrous ammonium sulfate solution. Ionization or relative depth dose has been determined for the electron beam using the thin-walled ionization chamber and unit-density dental wax as tissue-equivalent material.

A suitable photographic film has been found for use in the x-ray exposure range which is common for patient therapy. A scanner has been designed and fabricated for scanning the exposed film. The densitometer circuit attached to the scanner has been modified so that points on the film having the same density (which correspond to points receiving the same dose of x-radiation) are recorded automatically as the film is scanned. From these points isodose curves may be obtained.

To simulate a patient, a phantom has been fabricated from masonite using sections from "Cross-Section Anatomy" by Eycleshymer and Shoemacher. The phantom is constructed so that it comes apart in 1 cm. sections for insertion of the photographic film.

Several distributions for single field and rotational therapy have been completed and analyzed for accuracy.

Proposed course of project: An intensive study has begun in the development of isodose curves for rotational therapy. The effects on dosage distribution of partial rotation, displacement of center of rotation from the geometrical axis of the patient and axial dimensions of patients will be determined.

Other methods will be developed for particular types of therapy as the needs arise.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-660(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSIST-ANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17

(Use reverse and additional pages, if necessary)

Form No. CR-1 Calendar Year 1958
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-661(c)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Continuous Visual Monitoring of 2 Way Rotational Therapy
PROJECT TITLE

7. J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To achieve the highest degree of precision in beam orientation in both routine and research radiation therapy and especially as related to rotational therapy technic.

Methods employed: A closed television system employing special circuitry is employed which makes possible the continuous visualization of the x-ray therapy beam as it traverses the patient and tumor site.

(Use reverse and additional pages, if necessary)

10. Continued

The essential components of this system consist of a fluorescent screen upon which the x-ray beam impinges after passing through the patient and the tumor site, a television image tube focused upon the screen, an image intensification and contrast control circuit, and a viewing television screen at the control console outside of the treatment room. The instrumentation is elaborate for the interaction of the x-rays with the fluorescent screen material is very inefficient in terms of light production and image intensification by a factor of several thousand fold and contrast control are essential to the production of a visible image. The x-ray machine and the treatment chair have been so modified that all of the necessary positions controls are available at the operator's station outside of the treatment room. By means of these controls, the patient may be oriented in respect to the x-ray beam along two 90° axes and the x-ray beam itself may be raised or lowered. In this way, orientation of the beam to the tumor site is possible along the three major axes under direct visual control at the operating station.

Patient material: This technic for the precise control of beam orientation has been found to be adaptable to cases of pituitary tumor, cancers of the mouth and pharynx, intra-thoracic tumors including cancer of the esophagus, cancer of the uterine cervix, and cancer of the urinary bladder.

Major findings: Three general methods of beam orientation are available. The first of these is that of relating the beam to external anatomic landmarks. This method has the virtue of simplicity but it may be highly inaccurate. The next is radiographic control of beam orientation but this is time consuming and awkward and can only be done before or after the radiation procedure itself and it provides, essentially, only a check on what has been done and does not allow instantaneous corrections. The third and most precise method is that of fluoroscopic control described above. With this method adjustments may be made simultaneously with the radiation therapy and a continuous and very precise control can be maintained over beam orientation.

Proposed course of project: The procedure has been established as routine practice in the types of cases described above and its usefulness in other treatment problems will be explored.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-661(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,880	$3,140	$11,020	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-662(c)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The Effect of Ionizing Radiations on Advanced Carcinoma of the Urinary Bladder
PROJECT TITLE

7. J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To determine the clinical and objective responses of patients with very advanced carcinoma of the urinary bladder to ionizing radiations.

Methods employed: Appropriate patients (see below) are given radiation therapy to the bladder and to possible zones of extension in the pelvis by external radiation with a 2 mev x-ray beam. Multiple fixed portals or rotational technics are employed as may be indicated. Tumor dosage is of the order of 6000 roentgens delivered in 6 weeks through fairly large fields.

(Use reverse and additional pages, if necessary)

10. Continued

Patients are extensively studied by preliminary complete urological work-up including biopsy and are studied at intervals during and after the course of radiation therapy. Studies include response as determined by symptoms, evidence of urinary tract infection, changes in the cystographic appearance of the bladder, cystoscopy, and subsequent biopsy. Consideration is also given, where necessary, to appropriate surgical procedures to relieve urinary obstruction whether in the urethera or ureters.

Patient material: Only patients with far advanced carcinoma of the bladder have been admitted to this project. These patients have had previous partial cystectomy and all have recurrence of tumor in the bladder as demonstrated by biopsy and many of them have recurrence or metastasis of tumor into the pelvis as demonstrated by physical examination or as determined by laparotomy at the time of resection of the bladder. In other words, these are very unfavorable cases in which the usual modes of treatment, that is, electrosurgical dissection, radon implantation, partial cystectomy, and, in some instances, previous radiation therapy, had been used with failure to control the cancer.

Major findings: Some seven patients in the category described above have been treated on the project which is now only a few months old. As would be expected with the type of patient material described, complications have been frequent and there have already been deaths from urinary obstruction and pyelonephritis. Recurrent infection of the urinary tract has been frequent and constitutes a difficult clinical problem. One instance of suprapubic perforation of the bladder has occurred. On the other hand, about half the patients have had a clinically useful response and in some instances there has resulted virtually complete disappearance of symptoms and objective findings with a negative cystoscopy. In other words, there have been favorable responses in patients in whom the only surgical procedure possible would have been total cystectomy and excision of pelvic nodes with the disability attendant upon transplantation of the ureters.

Proposed course of project: To continue the study as outlined above and to consider, especially, the possibility of additional interstitial methods of radiation therapy subsequent to the completion of external radiation.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-662(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-663(c)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Carcinoma of the Esophagus Treated by 2 Mev Rotational Therapy
PROJECT TITLE

7. Philip Rubin, M. D., and J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To study the tolerance of normal structures and ascertain the dosage level that can produce cancericidal effects in treating carcinoma of the esophagus. In view of the poor results today reported in the literature using conventional x-ray techniques as well as radical surgical methods in which there is a high associated morbidity and mortality, 2 mev rotational therapy is being studied to determine its efficacy in treating this disease.

(Use reverse and additional pages, if necessary)

10. Continued

Methods employed: 2 mev rotational therapy using the Lumicon screen for control in the delivery of the high doses of irradiation. Pulmonary physiology studies and EKG's have been taken during the course of treatment as well as before and after to correlate effects with dosage.

Major findings: Five of eight patients have completed treatment with various dosage schedules ranging from 6000 r in 6 weeks to 10,000 r in 100 days. Of notable interest in this group is that the one patient who expired had no evidence of carcinoma at post mortem examination in the esophagus or elsewhere. There are three patients still living since the completion of therapy and of these, two show no evidence of carcinoma at the present time clinically and by x-ray examination. Although the followup is much too short to make any definite statements, it would appear that this method should be pursued further.

Proposed course of project: To continue.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-663(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,880	$3,140	$11,020	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-664(c)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Treatment of Carcinoma of the Cervix Treated by 2 Mev Rotational Therapy versus 250 KV Therapy
PROJECT TITLE

7. Philip Rubin, M. D., and J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To evaluate the role of 2 mev versus 250 KV irradiation in treating extremely advanced carcinoma of the cervix, clinical stage IV. It is felt that 2 mev rotational therapy will prove to be superior in palliating these cases and lower the complication rate because:

(a) it provides for a more uniform distribution and greater depth dose of irradiation

(Use reverse and additional pages, if necessary)

10. Continued

(b) lack of differential absorption in bone lessens danger of bone necrosis

(c) skin sparing effect allows for greater total dose

However, the extensive nature of the disease has necessitated large fields, particularly to include periaortic lymph node metastases and the risk of factitial changes in bowel and small intestine remains if cancerocidal doses are to be obtained.

Methods employed: 2 mev rotational therapy using Lumicon control.

Patient material: Extremely advanced carcinoma of the cervix patients, most of whom are clinical stage IV and have been found inoperable by even the standards of radical surgery and pelvic exeneration. Twenty-one patients have been treated in this study to date.

Major findings: During the past year, 13 patients have been treated with 2 mev rotational therapy, most of whom have been on a service basis. Palliation has been the objective in 12 of these and one of them was considered possible candidate for curative treatment. Also in this group, 3 fitted into other research programs in the department. In this group of 21 patients, one notes a definite increase in the survival time and efficacy of palliation in the patients treated with the higher type of irradiation. The conclusions we can reach with this limited study are (1) of the 8 patients treated with 250 KV irradiation, the maximum survival was 10 months, the average survival 4 months. (2) Of 13 patients treated with 2 mev irradiation, 9 are living, the maximum survival being more than a year and the average survival at the present time is 7 months. As these patients continue to survive the average survival period will lengthen during the following year.

Proposed course of project: To continue.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-664(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-665(c)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Study of Changes in DNA and RNA Following X-irradiation in Preparations of Cytologic Smears from Mucous Membranes
PROJECT TITLE

7. Philip Rubin, M. D. and J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. Albert Hilberg, M. D. and Sara DeWitt
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

The exact mechanism of cell injury and death that occurs following irradiation remains an enigma. Of the many areas in chemical cytology under exploration for an answer to this problem, nuclei acid metabolism is receiving a considerable share of attention. In the recent publication of "Biochemical Aspects of Basic Mechanisms in Radiobiology," edited by Dr. Patt -- a build-up of RNA occurs in the post-irradiation period. The exact mechanism for the increase in RNA is not apparent.

(Use reverse and additional pages, if necessary)

10. Continued

One theory suggests blockage of conversion of RNA into DNA. Another theory suggests that some RNA is inactivated but still physically present; the cell is then compelled to make new RNA, thus the total cellular RNA is actually increased after exposure to irradiation.

By histochemical staining techniques RNA and DNA can be differentiated and their employment in post-irradiation tissues has been limited to animal studies. MacCardle and Congdon have shown that the mitrochondria (rich in RNA) in hepatic cells respond promptly in nearly all mice exposed to total body x-irradiation of lethal magnitude. Briefly, certain alterations were noted in the mitrochondria 6-8 hours after injury. Comparative changes in the hepatic cells after histochemical staining show a globulated appearance (RNA) which increases with dosage and time. Twenty-four hours seems to be time of maximal effect with diminution following believed to be due to cellular degeneration in moribund animals.

Objectives: 'SR' and 'RR' changes in the basal cells of vaginal smears in patients with cancer of the cervix have been correlated with survival time by the Grahams. Since the 'RR' or radiation reaction is represented by an increased vaculization in the cytoplasm during the course of x-irradiation, it seems that this may be RNA. Accordingly, histochemical staining techniques have been adapted for this purpose with studies applied to the mucous membrane in the oral cavity as well as in the female genital region. It would be of interest to identify this vaculization which is seen in the parabasal cells since it may provide additional information as to the radiation response of tumors and tissues.

Patient material: Patients under treatment for various carcinomas of the oral cavity, nasopharynx and pharynx and uterine cervix.

Methods employed: Cytologic preparations are made and stained by various techniques. Slides are generally fixed in Carnoy's solution and stained for DNA and RNA with Methylene Green-Pyronin Method.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-665(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,880	$3,140	$11,020	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-666(c)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies in Secretory Sialography
PROJECT TITLE

7. Philip Rubin, M. D. and Byron Besse, M. D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: Secretory sialography has been applied in a wide variety of diseases of the salivary glands. One of the common problems faced clinically is the differential diagnosis and etiology of swellings about the parotid gland. This is particularly true of the Mikulicz's disease and Mikulicz's syndrome category. This method of studying the salivary glands may provide an index to differentiating these clinical entities.

(Use reverse and additional pages, if necessary)

10. Continued

Methods employed: Catherization of the salivary gland duct and injection of pantopaque in a retrograde fashion. This is followed by stimulation by means of a sialogogue to increase salivary flow and wash the contrast material out of the duct system.

Major findings: Different patterns have been found in patients with Mikulicz's disease and Mikulicz's syndrome roentgenographically enabling one to differentiate this group of cases. This has been correlated with biopsy material from these patients.

Patient material: Patients with various swellings about the parotid and submaxillary gland lesion which have fitted into the category of Mikulicz's disease or Mikulicz's syndrome. This includes a wide variety of patients with sarcoid, tuberculosis, lymphoma, leukemia and Hodgkin's disease. More than 40 such patients have been studied to date.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-666(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

(Use reverse and additional pages, if necessary)

Form No. C P-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI-666(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Philip Rubin, M. D. and Byron Besse, M. D., Sialographic Differentiation of Mikulicz's Disease and Mikulicz's Syndrome, accepted for publication in Radiology.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-667(c)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6.
PROJECT TITLE

7. Arnold L. Flick, M. D.
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: The objective of this study is to estimate the differential absorption ratio of Phosphorus-32 by the myeloma cell during simultaneous administration to the patient of Phosphorus-32 and Nilevar, an anabolic steroid.

Methods employed: Bone marrow and peripheral blood specimens were obtained from patients suffering from myeloma and receiving therapeutic

(Use reverse and additional pages, if necessary)

10. Continued

doses of Phosphorus-32 and Nilevar. The leukocytes of these specimens were separated from the erythrocytes and differential counts performed on the leukocytes. Radioactivity was then determined on representative leukocyte samples and a crude estimate made of the contribution to the radioactivity derived from the plasma cells in the specimen.

Patient material: The three patients used are people with multiple myeloma who were inpatients or followup patients of the Radiation Branch, National Cancer Institute.

Major findings: Within the order of magnitude of the error inherent in this experiment plasma cells were not found to take up more radioactivity than adjacent normal marrow cells in patients receiving Phosphorus-32 and Nilevar.

Significance to Cancer research: It is anticipated that favorable response of a patient with myeloma to the use of Phosphorus-32 will occur only with sufficient concentrations of the radioactivity in or about the myelomatous cells to cause the destruction of the myeloma, without there being simultaneous concentration of radioactivity in the normal marrow of such degree as to produce irreversible marrow aplasia. Although such a response might well be gauged by the long term study of many patients, this effort was undertaken to see whether experimental evidence for a favorable plasma cell DAR could be obtained with the methods outlined above, thus obviating the need for an extended and perhaps fruitless study of many patients. The results of this study fail to show such a favorable DAR. The estimated order of magnitude of the errors in this study are felt to be between 500 and 1000 per cent. It is to be noted that in other areas where differential absorption ratios are taken advantage of in the treatment of cancer such ratios are much above 10 and in the case of the thyroid gland are said to be in excess of several hundred to a thousand. Hence, it is felt that the sizeable errors here are none the less small enough to have permitted the experimental method to have demonstrated a clinically significant DAR.

Proposed course of project: Since the project of the treatment of myeloma patients with Phosphorus-32 and Nilevar has been at least temporarily discontinued there are no further plans for this project.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-667(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$13,140	$5,220	$18,360	1	2/3	1 2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	2/3	1 2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-668(c)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Human Radioisotope Studies
PROJECT TITLE

7. Kirkland C. Brace, M. D.
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To test various radioisotopes and determine their usefulness in the diagnosis and the treatment of cancer.

Methods employed: Doses of Iodine-131 and Iodine-132 are given by mouth to patients with cancer of the thyroid. Small doses are used for iodine uptake studies and for scanning with the scintillation counter to determine the extent of the primary cancer and metastasis.

(Use reverse and additional pages, if necessary)

10. Continued

Massive doses are administered for treatment of metastatic carcinoma of the thyroid which cannot be treated by other means. Iodine labeled rose bengal is administered intravenously to patients with suspected liver metastasis in order to obtain an outline of the liver with the scintillation counter.

Major findings: Iodine-131 has proved most satisfactory for iodine uptake studies and for determining the extent of the thyroid cancer. Large doses have proved very useful in the long term control of thyroid metastasis. The use of rose bengal to determine the size and contour of the liver appear very promising and should aid in the detection of tumor metastasis in the liver. Iodine-132 has been tested for use in short uptake studies but has been found to be unsatisfactory because of the variable absorption from the GI tract and contamination with tellurium.

Significance to Cancer research: The radioisotope studies offer considerable aid both in the diagnosis and treatment of cancer.

Proposed course of project: The thyroid cancer and liver tumor studies will be expanded as suitable case material becomes available.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-668(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,890	$3,140	$11,030	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No Entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-770(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Radiation Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Radiation Therapy Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Screening
PROJECT TITLE

7. J. Robert Andrews, M. D.
PRINCIPAL INVESTIGATOR

8. Philip Rubin, M. D.
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To explore program and other investigative possibilities by the study of unusual types of cancer, unusual manifestations of cancer or cases presenting with unusual technological problems.

Methods employed, patient material, procedures and protocol course: Patients in the Screening category [illegible] in report NCI-650(C) entitled Radiation Therapy Service.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-770(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,880	$3,140	$11,020	2/3	1/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2/3	1/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No entries for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

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Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-7018(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Nutrition and Metabolism Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Metabolism of Nitrogen, Calcium, Phosphorus and Electrolytes in Metastatic Prostatic Carcinoma
PROJECT TITLE

7. Donald M. Watkin, M.D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

This project is being incorporated under NCI-6-7211(C), Nutrient and Energy Metabolism in Subjects with Malignant Disease.

Brief Summary: Two patients were admitted, one of whom died shortly after admission. The other patient was admitted for a 24 day follow-up balance study, the third since discovery of the disease three years ago. In this study, the efficacy in maintaining positive nitrogen and calcium balances of stilbestrol was demonstrated.

(Use reverse and additional pages, if necessary)

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INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-701B(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,590	$35,200	$46,790	1/2	2/3	1 1/6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	420
FY'57	1/2	2/3	1 1/6	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

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Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-702(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Metabolism Service
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Metabolic Study of Serum Proteins
PROJECT TITLE

7. John L. Fahey, M. D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

Section B is being conducted in active collaboration with Drs. H. A. Sober and E. A. Peterson of the Laboratory of Biochemistry, NCI., who are developing methods of protein separation and identification.

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

A. Electrophoretic Studies of the Serum Proteins in Neoplastic Disease States.

Objectives: The objective of this project is to electrophoretically quantitate the serum protein changes occurring in neoplastic states and to correlate these changes with specific aspects of the clinical status of the patients.

Methods Employed: Quantitative zone electrophoresis was utilized during the year. Systematic follow-up of patients admitted to the Metabolic, Acute Leukemia, and Solid Tumor Services was carried out so that serum protein data can be correlated with a clinical evaluation of the type and extent of disease, disease activity, nutritional

(Use reverse and additional pages, if necessary)

Methods Employed (cont'd)
state of the host and therapy employed. Sequential observations in individual patients are an important aspect of this study.

Patient Material: Patients admitted to the Clinical Center for other projects were utilized in this study.

Major Findings: Long term patient observation is continuing. Marked quantitative changes were found in the abnormal globulin of several but not all patients with Multiple Myeloma receiving experimental therapies.

General assistance was rendered to all clinical groups for evaluation of abnormal serum proteins.

Abnormal "myeloma" globulins were demonstrated for the first time in the serum of mice bearing plasma cell tumors. This was done in collaboration with Dr. Michael Potter, Leukemia Studies Section, Laboratory of Biology, NCI.

Significance to Cancer Research: Delineation of the sequence of plasma protein changes in neoplastic disease is needed, for this is an aspect of tumor-host relationship which lends itself to quantitation. Also, the effects upon the serum proteins of anti-tumor therapeutic regimens is generally unknown.

Proposed Course of Project: Emphasis will continue to be upon serial determinations throughout the course of illness in the patients studied, and upon correlation with clinical data. Evaluation of the effects of cortisone, similar steroids, and other chemical agents will continue.

B. Chromatography of Serum Proteins

Objectives: The objective of this project is to further characterize the serum proteins from normal and tumor-bearing patients by application of new methods of protein separation.

Methods Employed: Modifications of the chromatographic column techniques of Sober and Peterson (Laboratory of Biochemistry, NCI) have been employed in these studies. Individual protein components are identified by electrophoretic, spectrophotometric and chemical means and by testing for biologic activity.

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NCI-6-702(C)

Patient Material: Patients admitted to the Clinical Center for other projects were utilized in this study.

Major Findings: Improvements in methodology were achieved and application of chromatographic techniques to clinical material was initiated.

The identification, localization and separation of acid phosphatase, alkaline monophosphatase, alkaline diphosphatase, siderophilin, and the thyroxine-binding protein were carried out in normal and/or abnormal sera. Studies carried out in collaboration with Dr. Andrew Peacock appear to establish the alkaline mono- and di-phosphatases as separate and distinct enzymes.

Significance to Cancer Research: The alteration of body protein metabolism in neoplastic states is reflected by serum protein changes. These have as yet been only grossly defined by other analytical means. Further identification and characterization of the serum proteins themselves are necessary in order that the causes of protein abnormality may be studied.

Proposed Course of the Project: Additional effort will be devoted to the development of a modified, more rapid adaptation of the present column chromatographic technique. Studies are planned to further identify the composition of fractions obtained by chromatographic separation of the serum proteins. Application of this technique to the characterization of the serum proteins from patients with a variety of neoplasms and states of disease activity will be undertaken.

C. Evaluation of Protein Metabolism by Means of Isotopically Labelled Amino Acids.

The need for further development of protein fractionation procedures has delayed the activation of this project. However, in the coming year a study of the formation of myeloma globulin in the mouse plasma cell tumor, x5563, will be initiated. In addition to the opportunities in this work in animals, such a study will be a very useful preliminary to clinical studies.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-702(9)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$34,260	$94,700	$128,960	2 2/3	1	3 2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3 2/3	1	4 2/3	1100

13. BUDGET ACTIVITY:

RESEARCH	☒	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

A. The Acute Leukemia, Solid Tumor and Surgical Services of the NCI have made available blood samples from patients admitted [illegible] study on these services and have collaborated generously in providing the clinical information necessary for adequate interpretation of the serum protein data.

B. Drs. A. L. Baker and [illegible] Peterson of the Physical-Chemistry Section of the Laboratory of Biochemistry, NCI, have been responsible for the development of this chromatographic method and are actively collaborating in the application of this technique to clinical studies.

[illegible] entries for items 15, 16, and 17.

(Use reverse and additional pages, if necessary)

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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-702B(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Metabolism
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Measurement of albumin turnover and total exchangeable albumin in patients with cancer, control patients with weight loss and volunteer normal subjects.
PROJECT TITLE

7. J. L. Steinfeld, M.D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Project NCI-6-702B(C) is completed. The final findings are in accord with those presented in the 1955 Annual Report; namely, that the low plasma albumin concentrations in those patients with advanced cancers studied in this project are associated with a normal plasma volume and a decreased total exchangeable albumin. The rate of albumin turnover is in the low normal range, thus indicating along with the other reported data that the findings in the cancer patients result from a decrease in albumin synthesis rather than an increase in albumin breakdown.

(Use reverse and additional pages, if necessary)

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-702B(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,960	$1,570	$5,530	1/3	1/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entires for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-703(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Metabolism
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Estimation of Red Cell Survival in Patients with Advanced Neoplastic Disease Before and After Treatment
PROJECT TITLE

7. J. L. Steinfeld, M.D., Paul Schmidt, M.D., Hugh Chaplin, M.D. and Arnold Flick, M.D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**
NCI Project 341, Drs. V.Price and R. Greenfeld.

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: This project can be divided into several parts:

(1) The measurement of red cell survival in cancer patients in various stages of the neoplastic process and in relationship to therapy.

(2) A. The determination of the distribution of Cr^{51} in tumor and other tissues at operation or at autopsy during or following a red cell survival study.

B. The determination of the distribution of Fe^{59} (given intravenously as Fe^{59} labelled red cells)

(Use reverse and additional pages, if necessary)

Calendar Year 1956

NCI-6-703(C)

Objectives (cont'd): in tumor and other tissues at operation or at autopsy.

C. The determination of the distribution of doubly labelled (Fe^{59} and Cr^{51})red blood cells using pulse height analysis - otherwise as in A and B above.

3. Survival times of red cells stored and frozen at -45 to -23°C. was measured.

Methods Employed: Standard methods for determination of Cr^{51} and Fe^{59} Red Cell Survival Studies will be employed. Blood and tissue studies will be carried out in a well scintillation counter with or without use of a pulse height analyzer. Stable nitrogen and/or phosphorous will be measured on all tissue samples studied.

Patient Material:

Adult Males	3
Adult Females	3
OPD Visits	20

Major Findings: (1) Red cell survival as measured by this technique is less than normal in patients with advanced cancers.

(2) This is a proposed study in collaboration with Dr. Arnold Flick.

(3) Red cells frozen and stored for 6 months at -45°C have a life span similar to that of red cells stored for less than 10 days in blood banks in the usual manner. Blood stored at -20°C has a considerably shorter survival.

Significance to Cancer Research: Many techniques have now been used to show that the anemia of some cancer patients is due to a shortened red cell life span.

It will be of considerable importance to determine the fate in cancer patients of the several components of red blood cells which are susceptible to study through radioisotope labelling.

The fact that red cells can be kept viable and healthy for months at the sub-zero temperatures is of considerable importance from the point of view of disaster planning, etc. or having available stored rare blood types for clinical use.

Proposed Course of Project: In line with objectives.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-703(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$10,740	$20,590	$31,330	2/3	2/3	1 1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	250
FY'57	2/3	2/3	1 1/3	

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-704(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Metabolism
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The Influence of Delta-Aminolevulinic Acid and Some of its Analogues on Tumor Growth.
PROJECT TITLE

7. Donald P. Tschudy, M.D.
PRINCIPAL INVESTIGATOR

8. Martin Schick, M.D.
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: A. To develop compounds which interfere with porphyrin biosynthesis and study their effects on growth.

B. To study the effects of certain drugs and vitamins on prophyrin biosynthesis.

C. To study host-tumor interrelationships from the standpoint of porphyrin synthesis in liver and red cells.

Methods Employed: A. Synthetic organic chemistry.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

NCI-6-704(C)

Methods Employed (cont'd)

B. Animal pharmacology and animal antitumor studies.

C. Bacterial growth rate methods.

D. Quantitative enzyme determinations.

Patient Material: Patients have not been studied as yet, but porphyrin synthesizing enzymes will be studied in patients in the near future.

Major Findings: A. A number of compounds have been synthesized and most of these have been studied for their effect on the growth rate of three bacterial species (obligatory aerobe, facultative anaerobe, obligatory anaerobe). Several are significant inhibitors of growth and three stimulate growth to varying degrees, one being a very powerful growth stimulator.

These compounds are being studied for their effects on sarcoma 180, leukemia 1210 and carcinoma 775, but are not yet complete.

B. The quantitative method for measuring the enzyme which synthesizes porphobilinogen (aminolevulinic acid dehydrase) has been applied to a number of tissues including tumors and organs of tumor bearing animals. The enzyme activity in livers of tumor bearing animals is markedly reduced.

Significance of Program to Cancer Research: Studies of the effects of the compounds on tumor growth are of chemotherapeutic interest. Their effects on growth rates in bacteria with reference to effects on aerobic and anaerobic metabolism are of considerable interest because of the basic question of the relative importance of increased anaerobic metabolism to carcinogenesis and tumor growth.

The finding of a reduction of the porphobilinogen synthesizing enzyme in the livers of tumor bearing animals is of importance in tumor-host relationships.

Proposed Course of the Project: A. To study some of the mechanisms of bacterial growth stimulation.

B. To study the effects of certain drugs (mainly anti-cancer drugs) and vitamins on porphyrin synthesis in animals and humans.

Proposed Course of the Project (cont'd)

C. To study the mechanism whereby the enzyme level is reduced in the liver of the tumor-bearing host.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-704(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$17,810	$7,100	$24,910	1-1/2	1 1/2	3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1 1/2	1 1/2	3	0

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for items 12, 13 and 14.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-5-706(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Solid Tumor Chemotherapy
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Study of the value of a skin test using polysaccharide in cancer and non-malignant disease.
PROJECT TITLE

7. John H. Tuohy, M.D. and Murray Shear, Ph.D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

This project was designed to confirm the published results of Japanese investigators. Its design involved the intra-cutaneous injection of a bacterial polysaccharide solution prepared by the Laboratory of Chemical Pharmacology, NCI, into patients with previously diagnosed malignant disease. In addition this same method was to be applied to patients with non-malignant diagnoses for control purposes.

The described activity has been dormant for the past year and it is requested that it be discontinued due to the separation from service of one of the investigators (J. H. Tuohy).

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-5-706(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	0	0	0	0	0	0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	0	0	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None

No entries for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-5-707(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Solid Tumor Chemotherapy
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Evaluation of Blood and Plasma Water Content in Patients with Malignant Neoplasms
PROJECT TITLE

7. John H. Tuohy, M.D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Using the Karl Fischer reagent, an attempt was to be made to determine moisture content of blood, plasma and other tissues in patients with malignant and non-malignant disease.

Since this project has been in abeyance for the past year, and since the investigator is being separated from the service, it is requested that this project be discontinued.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-5-707(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	0	0	0	0	0	0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	0	0	0

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-7-708(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Dermatology
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Dermatologic Research
PROJECT TITLE

7. E. J. Van Scott, M.D., R. C. MacCardle, M.D., and R. P. Reinertson, M.D. and J. R. Andrews, M.D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):** A. Relationship of Junctional Activity in Pigmented Nevi to Development of Melanomata: E. J. Van Scott,M.

Objectives: To determine incidence of activity, functional or otherwise in nevi of patients with and without melanoma; to determine possible local factors in the skin which may induce malignant degeneration of nevi.

Methods Employed: Selected nevi to be excised from the backs of patients with and without melanoma. Histologic characteristics of nevi in each group to be compared.

Flat nevi of patients with and without melanoma to be transplanted intradermally in the same patient and re-excised subsequently.

(Use reverse and additional pages, if necessary)

Methods Employed (cont'd):

Changes in histologic character to be studied.

Flat nevi and normal epidermis of patients with melanoma to be transplanted into cutaneous metastases of melanoma in the same patient. Changes in histologic character to be studied.

Patient Material: Patients hospitalized in the Clinical Center or seen in Admissions and Follow-Up Clinic.

Major Findings: Histologic characteristics of 31 nevi from backs of patients without melanoma and of 73 nevi from backs of patients with melanoma suggest that nevi in both groups of patients are equally benign (or equally pre-malignant).

In two patients without melanoma, and in one patient with melanoma flat junction nevi have been transplanted intradermally and re-excised subsequently. Final interpretation of changes which have occurred in such nevi has not been made.

Significance to Cancer Research: Attempt at understanding factors leading to malignant degeneration of nevi.

Proposed Course of Project: Continuation as outlined above.

10. B. PROJECT DESCRIPTION: Histologic and Histochemical Anatomy of Certain Skin Lesions, as Correlated with Gross Anatomy: E. J. Van Scott, R. C. MacCardle, and R. P. Reinertson.

Objectives: To establish gross and microscopic anatomical changes which occur in the skin and its appendages under normal and pathological conditions.

Methods Employed: (1) Balsa wood models, 75 x normal size, of pilosebaceous units are reconstructed from serially sectioned biopsy specimens.

(2) Histological and histochemical observations of epidermis and appendages in health and disease.

(3) Microscopic examination of roots of manually extracted hairs.

Calendar Year 1956

NCI-7-708(2)

Patient Material: Patients hospitalized in the Clinical Center or seen in Admissions and Follow-Up Clinic.

Major Findings: (1) 38 models of pilosebaceous units have been constructed. Normal and pathological anatomical characteristics have been established. The histogenesis of the lesion of acne has been described.

(2) Histological and histochemical characteristics of the granular layer of the epidermis have been described.

The lesion of psoriasis, naturally occurring and experimentally produced, may histogenetically be accounted for by premature dehydration of epidermal cells.

(3) a. Data on the percentages of growing and resting hairs of the scalp have been obtained.

b. Quantitative morphological changes in the hair roots in response to varying doses of ionizing radiation have been established.

c. Morphological changes in hair roots in response to chemotherapeutic drugs have been observed.

Significance to Cancer Research: This study is directed toward understanding:

(1) factors controlling growth of the hair bulb and spontaneous normal destruction of portions of the hair bulb during catagen phase of growth cycle;
(2) certain phenomena occurring normally and pathologically within the epidermis during the birth-to-death course of epidermal cells;
(3) changes occurring in hair roots in response to agents used therapeutically against cancer (i.e., ionizing radiation and chemotherapeutic drugs).

Proposed Course of Project: Continuation as outlined above.

10. C. PROJECT DESCRIPTION: Amino Acid Content and Enzymatic Activity of Skin and Mucous Membranes: E. J. Van Scott

Objectives: Quantitative determination of certain amino acids and activity of arginase and possibly other enzymes in various layers of normal and pathological skin, and in constituent tissues of the hair root.

Methods Employed: Chemical methods for the determination of arginine, cystine, sulfhydryl, and arginase employed. Other chemical methods utilized to depend on indications for analysis of specific substances or metabolic activities.

Patient Material: Tissues from living human beings obtained from patients of Clinical Center.

Major Findings: Determinations of arginase activity, arginine, cystine, and sulfhydryl in water soluble and water insoluble fractions of a large number of epidermal tissues have been completed and are now to be evaluated and interpreted.

Significance to Cancer Research: Determination of patterns of amino acid composition and biochemical processes in epidermis and hair roots, tissues in which cell populations may be followed from birth to death, may give information regarding processes concerned in the normal destruction of living cells; extension of such information to the problem of destruction of cancerous cell populations to be considered.

Proposed Course of Project: Continuation as above, with greater emphasis on study of tissues of the hair root.

10. D. PROJECT DESCRIPTION: Therapy of Mycosis Fungoides with High Energy Electrons: E. J. Van Scott and J. R. Andrews

Objectives: Therapy of patients with mycosis fungoides in whom the disease is apparently limited to the skin, with high total doses of electrons emitted from van de Graaff generator.

Method Employed: Increasingly large doses of electron radiation are given to whole body surface. Attempts made to establish maximum tolerance doses and to administer such doses with view that these may be curative of the early disease. Frequent biopsies, photographs, and clinical laboratory procedures obtained during period of therapy.

Patient Material: Patients hospitalized in Clinical Center. (Total of 7 to date).

Major Findings: Prolonged periods of clinical remission of the disease have occurred in one patient. Maximum dose of electrons given to date has been 3,000 rad.

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NCI-7-708(C)

Significance to Cancer Research: Treatment of one form of cancer.

Proposed Course of Project: Continuation as outlined above.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-T-708(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$45,840	$54.80	$100.640	2 5/6	3 2/3	6 1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3	3 2/3	6 2/3	638

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-4-709-(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The Chemotherapy of Acute Leukemia
PROJECT TITLE

7. Dr. Emil Frei, III
PRINCIPAL INVESTIGATOR

8. E. J. Freireich, M.D., James Stengle, M.D.,
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: 1. To study in a definitive way the effects of known effective drugs on the course of acute leukemia.
2. To study the effects of these compounds on the host.
3. To discover new chemical compounds which can alter the course of acute leukemia.

Methods Employed: 1. Case Material: All patients referred to the Clinical Center with the diagnosis of acute leukemia are admitted to the study. In addition there are now three hospital units other than the NIH participating in the comparative chemotherapy study (See #14).

(Use reverse and additional pages, if necessary)

Methods Employed (cont'd)

2. Criteria: Objective criteria for the evaluation of disease activity have been developed and applied (See publications).

3. Pharmacology: In cooperation with the Laboratory of Clinical Pharmacology, pharmacologic disciplines are applied to the drugs tested. These studies are divided into three major categories:

A. Toxicity studies of compounds of potential antitumor activity given to humans for the first time.

B. Absorption, distribution and excretion studies of known and potential antitumor compounds.

C. Analysis of the influence of varying dosage schedules and routes of administration on antitumor activity and toxicity.

4. Experimental Design: In cooperation with biostatitians and the Laboratory of Clinical Pharmacology therapeutic trials were designed. These include:

A. Cooperative Therapeutic Trial designed to obtain among other things, quantitative information of the relative effects of antimetabolites of proved antileukemic activity in man.

B. Sequential Analysis in patients with acute leukemia of new drugs (such as the agent now under study, 6-azauracil), in an effort to discover new chemical compounds capable of influencing the course of the disease.

Major Findings: 1. Methotrexate administered every third day does not differ in therapeutic effect or toxicity from methotrexate administered daily in comparable total dose. Both schedules were administered in combination with 6-mercaptopurine.

2. The administration of methotrexate and 6-mercaptopurine in combination does not improve response in terms of frequency of remission, extent of remission, duration of remission or survival. However, a direct comparison was not made.

3. 6-azauracil is under toxicity and antileukemic trial. The preliminary results are as follows:

A. Toxicity is primarily neurological. EEG changes and somnolence occur regularly if therapy is increased to toxicity. The following have occurred much less commonly; bizarre motor phenomena (choreoathetoid movements, postural thalamic syndrome), abnormal psychic phenomena (excessive dreams, hallucinations), convulsions on withdrawal, and arthritis. With one exception all of the above have been reversible. There is considerable variation among patients in the total dose necessary to produce toxicity.

Major Findings (cont'd)

B. Antileukemic effect - 4 of 13 patients have had hematological improvement occur while on 6-azauracil. For the most part this was slight and transient and in the only case in which complete remission occurred complicated by previous 6-mercaptopurine therapy. In one case a transient partial remission occurred that was clearly related to 6-azauracil.

Significance to Cancer Research: Trial in humans of compounds with potential antitumor effect is of obvious importance. Quantitative comparative studies designed to detect slight but statistically significant differences in antitumor effect are essential to direction of drug development into profitable areas.

Proposed Course of Study: This study will always have as its major goal the finding, defining and improving of drugs with human antileukemic activity.

Continuing efforts will be made to improve various aspects of the methods described above.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NCI-4-709(C)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Criteria for Evaluation of Response to Treatment in Acute Leukemia, Blood, Vol. 9, No. 7, p, 676, July 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

None.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-4-709(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF.	OTHER	TOTAL
FY'57	$57,330	$231,400	$288,730	3 5/6	2/3	4 1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3 5/6	1 5/6	5 2/3	2700

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Department of Pediatrics
University of Buffalo
Buffalo, N. Y.

Roswell Park Memorial Institute
Buffalo New York

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-5-711(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Solid Tumor Chemotherapy
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Assay of Compounds for Antitumor Effect by Injection into Skin Metastases.
PROJECT TITLE

7. Clyde O. Brindley, M.D.
PRINCIPAL INVESTIGATOR

8. ---
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To develop a method of assay of compounds for antitumor effect in humans by the injection of these materials into cutaneous and subcutaneous tumor masses.

Methods employed: Needle injection of the tumor masses; determination of antitumor effect by caliper measurement, comparative photographs and histological study of the injected lesions.

Patient material: None (see project 712(C))

Major findings: None

(Use reverse and additional pages, if necessary)

Significance to Cancer Research: A method whereby many agents could be screened for antitumor effect in human tumors would be useful. The direct injection of palpable tumor masses with agents being tested might be developed into such a method.

Proposed Course of the Project: During the past year patients with subcutaneous and cutaneous measurable lesions which would have been suitable for this study have been entered in another project (712-3). When such patients again become available it is planned that this study will be resumed.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-5-711(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,410	$11,460	$18,870	2/3	1/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2/3	1/3	1	133

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entry for items 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 5-712(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Chemotherapy
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

A. Controlled Studies in the Chemotherapy of Solid Tumors.

6. B. Chemotherapeutic Agents in Human Solid Tumors
PROJECT TITLE

7. C. O. Brindley, M.D., Bruce I. Shnider, M.D., and J. F. Tuchy, M.D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):** A. Controlled Studies in the Chemotherapy of Solid Tumors.

Objectives: (1) To set up an experimental design for a number of chemotherapy trials and gain experience with this design, so that a protocol could be formulated for a cooperative comparative study.

(2) Cooperate with several other institutions in formulating a final protocol and in carrying out a randomized controlled comparative study of a standard and a test chemotherapeutic agent in certain human tumors.

(3) Determine the relative effectiveness of these two agents in these human tumors.

Methods Employed: (1) Selection of patients having specific tumor types, measurable disease and prior treatment status according to a set protocol.

(2) Administration of the test and standard agents according to a set protocol.

(3) Assay of antitumor effect by direct caliper measurement of palpable tumor masses or measurement of tumor masses as shown on standardized X-ray films.

(4) Submitting these objective tumor measurements to statistical analysis and using these data as evidence of drug effect on the tumors.

Patient Material: Patients with Hodgkins' disease, carcinoma of the lung and breast and patients with melanoma are considered for the study.

Only patients with criteria of measurable disease and prior treatment status which satisfies the protocol are accepted for the study.

Major Findings: In the preliminary study, which was carried out on 30 patients using Mechlorethamine (nitrogen mustard) as standard drug and triethylene thiophosphoramide (Thio TEPA) as test drug, information was obtained which was essential in formulating the final protocol for the cooperative study. This included the drug dosage schedules, measurability of tumor masses and the techniques for carrying out these measurements, and methods of reporting the data obtained. On the basis of this information the members of the cooperating groups have drawn up a protocol for and are now engaged in carrying out the controlled study on nitrogen mustard and Thio TEPA. There have been or are now 30 patients from the NCI in this cooperative study. The cooperative group has studied 132 patients up to this date (Dec. 1, 1956).

Significance to Cancer Research: This is an effort to transfer the experience in experimental therapeutics in other fields of medicine to the study of chemotherapeutic agents in malignancy. The cooperative endeavor of which this project is a part has served as a pattern for studies by several other groups cooperating in the study of cancer chemotherapy. There is hope that in

Significance to Cancer Research (cont'd): this way drugs showing promise in more preliminary studies can be carefully and rapidly evaluated in a large number of human cases.

Proposed Course of the Project: The trial study using Mechlorethamine (nitrogen mustard) as standard drug and triethylene thiophosphoramide (Thio TEPA) as test drug is now being carried out in cooperation with five other institutions. It is planned that this trial study will be continued until statistically significant results are obtained by the cooperating group.

10. B. PROJECT DESCRIPTION: Preliminary Studies of Chemotherapeutic Agents on Solid Tumors.

Objectives: To test agents which have shown promise in other studies (animal screening and initial human trials) for toxicity, tolerated human dosage levels and evidence of antitumor activity in human solid tumors.

Methods Employed: Drugs are tested for toxicity by their administration in increasing dosages or for increasing periods of time at a fixed dose until signs of toxicity appear. Antitumor effect is determined by direct measurement of tumor masses, their measurement on standardized X-ray films or by similar observations which can be correlated with tumor growth. The riboflavin analogues are studied by photofluorometric techniques to measure plasma and urine concentrations of the drug prior to and following administration. Chromatography is used to determine the metabolic products of the analogues. Bioassays are utilized to study the biological activity of the products.

Patient Material: These are included under A. Patients participating in part A have been included in this study upon completion of their participation in the cooperative comparative trial.

Major Findings: Fairly complete toxicity data and some information on antitumor effect in humans has been obtained with the following agents:

(1) 6-Azauracil

Calendar Year 1956

NCI-5-712(C)

Major Findings (cont'd):

(2) Sodium-6,7,-dimethyl-9-isoalloxazine ethyl succinate (U-6538) (riboflavin analogue).

Agent (1) has marked central nervous system toxicity at dosage levels which vary widely from patient to patient. Agent (2) crystallizes out in the urine at a blood level which is similar for all the patients tested. Neither agent has shown significant antitumor effect in the human solid tumors tested so far. A large part of the metabolic products of agent (2) are not biologically active riboflavin antagonists.

Significance to Cancer Research: A logical part of a drug development program and sequel to animal screening is a rapid preliminary method for the assay of an agent's toxicity and probable antitumor activity in humans. It is a necessary preliminary to the final critical evaluation of any chemotherapeutic material.

Proposed Course of the Project: As agents are furnished from intramural and outside sources they will continue to be evaluated in this manner.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-5-712(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$87,890	$351,920	$439,900	6	1	7

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	7	1	8	4100

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15.NCI-5-712(C)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

1. Participant Panel on Cancer Chemotherapy, Montgomery County (OHIO) Cancer Control Society, Dayton, Ohio, October 3, 1956. (Dr. John H. Tuohy)

2. Speaker, Meeting, Arlington County (VIRGINIA) Medical Society, November 18, 1956. (Dr. John H. Tuohy)

3. Speaker on program of Freedman's Hospital, Former Interns and Residents Association. Cancer Chemotherapy at NCI, June 7, 1956. (Dr. Bruce I. Shnider)

4. Lecture to Radiological Society of North America, Chicago, Ill., Dec. 7, 1956. (Dr. C. O. Brindley)

5. Lecture to 3rd National Cancer Conference, Detroit, Michigan, June 6, 1956. (Dr. C. G. Zubrod)

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-5-714(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on Metabolism of Blood Cells and Blood Forming Organs
PROJECT TITLE

7. James M. Stengle, M. D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS): A. Iron Metabolism

Objectives: To compare the iron metabolism of the patient with malignant neoplastic disease with the normal; to study the question whether the leukemic cell or solid tumor cell is competing for iron at the expense of normal body requirements and is thus contributing to the anemia characteristic of these diseases.

Background: Recent work in the National Cancer Institute indicates that certain mouse tumors have a high iron content. It is commonly noted in bone marrow observation that stainable iron is lacking during an acute exacerbation of leukemia but may reappear during remission. It has been reported (Simmons and Everett) that normal rat leukocytes incorporate

(Use reverse and additional pages, if necessary)

Background (cont'd)
considerable amounts of radioactive iron administered orally or parenterally.

Methods Employed: In leukemic patients serial serum iron and leukocyte iron determinations will be made and correlated with hemoglobin and leukocyte counts.

In vitro studies of normal and leukemic leukocyte uptake of radioactive iron will be made.

In solid tumor patients serum iron and hemoglobin values will be followed and surgical and autopsy specimens analyzed for iron content.

Some of the above work will be carried out in cooperation with Dr. Arthur Schade of the National Institute of Allergy and Infectious Diseases.

B. Temperature and Hydrogen Ion Concentration Measurements of Bone Marrow.

Objectives: An instrument has become available which makes it possible to measure the temperature of the bone marrow accurately and quickly. Since the bone marrow is a well insulated and actively metabolizing organ it is of interest to accumulate temperature data in leukemia during exacerbation and remission and to observe the influence if any of the several chemotherapeutic agents.

A newly designed pH electrode makes a direct measurement of aspirated bone marrow feasible. The mechanism by which iron is removed at the site of utilization from the serum iron carrying protein, siderophillin remains unknown. At normal blood pH it is tightly bound, but reduction of pH into the acid ranges in vitro frees the iron from siderophillin. It seems possible that local tissue hydrogen ion concentration may be of interest in this respect.

Methods Employed: Temperatures will be measured using a Waters thermister equipped with needle probes which will be inserted into the bone marrow cavity through the standard bone marrow needle.

Hydrogen ion concentrations will be estimated on samples aspirated directly into a Beckman reservoir electrode chamber.

Calendar Year 1956

NCI-5-714(C)

C. Nicotinic Acid

Objectives: We propose to study the role of nicotinic acid in the metabolism of iron in normal and leukemic individuals. It is known that administration of nicotinic acid to a normal individual markedly increases the concentration of iron in the serum. The source of the iron is not known. The nature of the anemia accompanying leukemia seems inadequately explained. There is every evidence that leukemia is accompanied by profound metabolic disturbances. The study of nicotinic acid effects offers an approach toward clarification of these disturbances.

Methods Employed: Leukemic patients will be given parenteral and oral doses of nicotinic acid and the effects on serum iron, bilirubin, reticulocyte count and hemoglobin measured.

Animal studies using dogs on nicotinic acid deficient diets are in progress.

D. Studies on respiration and glycolysis of human leukemic cells in vitro.

Objectives: It is proposed to study the respiration characteristic of leukemic cells and to compare with normal leukocytes, and secondly to study the effects of the various chemotherapeutic agents on respiration and glycolysis.

Methods Employed: Techniques for the separation of leukocytes from erythrocytes are as yet imperfect. Sedimentation methods employing fibrinogen, dextran and gelatin are being investigated. The method described by Kline employing a "swimmer" for the purpose of separating the three major cell types will also be studied. Attempts will be made to separate granulocytes from lymphocytes by employing the adhesive property of granulocytes.

For the metabolic studies Warburg manometers will be used. This work is to be done in collaboration with Dr. Dean Burk of the Laboratory of Biochemistry, NCI.

Significance to Cancer Research.
To obtain an understanding of leukemia and cancer further knowledge of the metabolism of the malignant cell is needed. There are evidences of profound metabolic changes in patients with malignant disease. The above projects are intended to shed new light on questions in these areas.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards and Publications 15. NCI-5-714(C)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Stengle, James M., and Schade, Arthur L., Dirunal-Nocturnal variations of certain blood constituents in normal human subjects: plasma iron, siderophillin, bilirubin, copper, total serum protein and albumin, haemoglobin and haematocrit, British Journal of Haemotology, January, 1957.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956

None

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-5-714(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,960	$1,570	$5,530	1/3	1/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-4-715(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The Mechanism and Management of the Hemorrhagic Disorder Associated with Acute Leukemia
PROJECT TITLE

7. E. J. Freireich, M.D.
PRINCIPAL INVESTIGATOR

8. Emil Frei, III, M.D.
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: a) To identify factors responsible for precipitating gross hemorrhage in already thrombocytopenic patients.

b) To determine the mechanism of action of fresh blood transfusion in alleviating thrombopenic bleeding.

c) To improve the therapy of thrombopenic hemorrhage.

Methods Employed: Clinical observation of effects of therapy on bleeding utilizing a double blind technique. In vitro studies of antithrombin activity, tissue thromboplastin activity, and blood thromboplastin activity.

(Use reverse and additional pages, if necessary)

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Patient Material:

Patients on the Leukemia Service who develop clinical hemorrhage are utilized for study.

Major Findings: a) In a double blind comparative study, fresh blood was found to be definitely more effective in alleviating hemorrhage than was banked blood. Banked blood had no significant effect on hemorrhage, while fresh blood had a clinically detectable effect in over 70% of the instances where it was used.

b) Methods have been developed for the measurement of antithrombin activity. Bovine thrombin showed greater stability in human plasma than human thrombin. Moreover, thrombin which is generated in native plasma is less stable than any purified thrombin preparation tested to date.

c) Brain tissue obtained at autopsy from acute leukemia patients has been assayed for over-all thromboplastic activity, and found to be within control range. Thus tissue factors could not thus far be implicated in precipitating clinical hemorrhage.

d) Acute leukemia patients with clinical hemorrhage have been examined for clotting defects other than thrombocytopenia. No major defects have been identified in any patient to date. Screening studies include correction of platelet deficiency in vitro with the addition of normal platelets. In all patients studied to date this has completely corrected the clotting defect in vitro.

Significance to Cancer Research: Hemorrhage ranks with infection as the two major causes of death in acute leukemia. Moreover, irradiation as well as many chemotherapeutic agents used in the treatment of leukemia and other tumors result in thrombocytopenia and hemorrhage. At present no effective prophylaxis or therapy for thrombocytopenic bleeding is available. Any effective therapy of thrombopenic bleeding could greatly prolong life and possibly improve the effectiveness of other therapeutic agents in these patients.

Proposed Course: The effectiveness of fresh blood transfusion in alleviating thrombopenic bleeding has been demonstrated. We plan to attempt to

localize the effective agent in fresh blood by determining whether fresh blood without platelets has any effect on thrombopenic hemorrhage. In the event that platelets per se can be shown to be the effective agent, we will investigate other methods for concentrating and storing platelets such as freeze-drying.

Studies of precipitating factors will be continued. Antithrombin activity will be assayed in patients before and after clinical hemorrhage. Studies of other tissue and blood factors such as thromboplastin inhibitors will be undertaken.

Attempts to localize the phosphatide in platelets which is the active component, and to correct the defect of thrombopenia with soluble plasma constituents such as phosphatides will be made first in vitro and if indicated in vivo.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-4-715(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,880	$4,750	$16,630	2/3	1 1/3	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2/3	1 1/3	2	0

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. CRP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-4-716(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Nutritional Aspects of the Therapy of Acute Leukemia
PROJECT TITLE

7. E. J. Freireich, M.D. and Emil Frei, III, M.D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine the effects of nutritional factors contained in animal tissues on the course of human acute leukemia.

Methods Employed: Various animal tissues are emulsified, analyzed for chemical composition and presence of infectious agents, and if tolerated by experimental animals, are administered to acute leukemia patients. The course of the disease is followed by clinical observation of the patient and various hematological determinations on blood and bone marrow.

Patient Material: Five (5) acute leukemia patients have been tested to date.

(Use reverse and additional pages, if necessary)

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Major Findings: Emulsified material has been prepared from hog tissues and stored at -50°C. This material was fed to two (2) dogs for seven (7) days without ill effect, extensive bacterial examination failed to disclose any significant pathogens. The material was then given by stomach tube to four (4) patients and by mouth to one (1) patient. In the first patient studied it was found that it was extremely difficult to finely emulsify the material, requiring a very large bore stomach tube for administration. We were unsuccessful in attempting to mask flavor and administer the material per os in one patient. Of the five (5) patients treated, two (2) had onset of G. I. bleeding during administration, two (2) had definite abdominal cramps, one (1) patient developed aspiration pneumonia, probably related to passing the tube. In only one (1) patient was it given for fourteen (14) days without complication. None of the patients showed detectable improvement in their leukemia.

An experiment to test these factors was performed in DBA mice. Mouse tissue was emulsified and freeze dried, and the experimental animals were then fed the material. A group of 24 litter mate controlled DBA mice were studied in three groups. Group one received normal diet, group two received DBA mouse tissue and group three received Swiss general purpose mouse tissue, not pure strain. The animals were fed these diets from weaning (3-4 weeks) for seven (7) weeks. They were then inoculated IP with 2,800 cells of L-1210. All animals died at 10-13 days, and no positive effects of treatment were demonstrated.

Significance to Cancer Research: The major approach to the therapy of neoplastic diseases has been and continues to be the use of cytotoxic agents. As a new approach, based on the assumption that the neoplastic cells are capable of maturing and returning to normal, nutritional non-cytotoxic factors will be used in the treatment of acute leukemia. The discovery of a factor or factors that would promote the maturation of leukemic cells would have immense significance.

Proposed Course of Project: We do not plan to continue these investigations with animal tissues at this time.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-4-716(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,880	$4,750	$16,630	2/3	1 1/3	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2/3	1 1/3	2	0

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-4-717(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Infections, Fever, and Host Resistance in Acute Leukemia
PROJECT TITLE

7. Dr. Claude Forkner
PRINCIPAL INVESTIGATOR

8. Dr. John Utz, NMI; Dr. David Rall, Dr. Emil Frei, NCI
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: 1. To obtain data relating to the natural history of fever and infections in acute leukemia.

2. To define the mechanisms of impaired host resistance to infection in acute leukemia.

Methods Employed: Fevers of known and unknown origin have systematically been studied according to a previously defined protocol in over 30 patient with acute leukemia. Comprehensive bacterial and viral data have been collected and analyzed in conjunction with a group in the National Microbiological Institute.

(Use reverse and additional pages, if necessary)

Methods Employed (cont'd)

In order to define the capability of leukemic granulocytes to phagocytize opsonocytophagic determinations have been made in 11 patients. The antibody response to 6 challenge antigens has been measured in 11 leukemic patients and in 7 "normal controls." Alterations in serum proteins during the immunization procedure have been measured by paper electrophoresis.

Major Findings: Several units of this study are completed and either have been or are about to be submitted for publication. Results are as follows:

I. A. Fever and infection were studied with comprehensive bacterial, fungal and viral techniques in thirty-six consecutive patients with acute leukemia.

B. As a group, these patients were febrile for almost one-half of the total 2,614 patient-days. Only two patients had no febrile episodes during the period of observation. Of the ninety-two febrile episodes recorded, fifty-nine appeared to be related to infection. In the remaining thirty-three febrile episodes bacterial, viral, or fungal infection, antimetabolite hypersensitivity, and lesions in the hypothalamus could not be incriminated as a cause for fever.

C. Age and type of leukemia did not influence the frequency of febrile episodes. However, adults developed more febrile episodes related to proved or presumptive infection than did children.

D. The most common clinical illnesses associated with the proved infections were pharyngitis, pyelonephritis, and septicemia. On occasion, relatively avirulent bacteria were capable of causing serious clinical infection. The most frequently encountered organisms were E. coli, M. pyogenes var. aureus, coagulose positive, and Ps. aeruginosa. Infections due to Ps. aeruginosa were the most difficult to treat.

E. Survival of the infection was common, in the absence of bacteremia, if intensive and appropriate antimicrobial therapy was employed. Nevertheless infection remained a leading cause of death in those patients who died during the study.

F. The majority of the presumptive infections were related to infections of the respiratory tract.

Major Findings (cont'd)

G. In the initial evaluation of the febrile leukemic patient, it is to be emphasized that a negative physical examination does not exclude infection as a cause for fever in a patient with acute leukemia.

H. Of the fourteen fevers of unknown origin which persisted after two weeks of antibiotic therapy, eight eventually terminated in serious bacterial infection. Appropriate management for persistent fever of undetermined origin requires continual reappraisal to insure that a superimposed infection, masked by preceding unexplained fever, has been detected.

I. The pathogensis of fever of undetermined origin and the mechanism of impaired resistance to bacterial infections in acute leukemia are complex problems which are being investigated further.

II. Antibody production and plasma protein changes following viral and bacterial antigen do not differ appreciably between acute leukemia patients and controls.

III. Phagocytosis by granulocytes is normal in acute leukemia. Leukemic cells have no phagocytic properties when incubated with brucella organisms.

IV. The absolute polymorphonuclear count falls in the period immediately preceeding infection.

A series of three deaths within a week due to Pseudomonas septicemia on a ward of 10 patients with acute leukemia has stimulated investigation of the incidence, pathogenicity, and clinical course of these infections in the clinical center in general and the leukemia service in particular. To date, over a period of about 2 years, approximately 18 septicemias due to this organism have occurred, 12 of which have been in patients with acute leukemia. All the cases have been recropsied and related pathological data will be carefully investigated. It is hoped that with a careful evaluation of the circumstances under which these infections occurred, more may be learned regarding the prevention and management of such cases.

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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NCI-4-717(C)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Silver, R. T., Utz, J. P., Frei, E., Zubrod, C. G., and McCullough, N. B., Fever, Infection, and Host Resistance in Acute Leukemia. To be submitted to A. J. Med.

2. R. T. Silver, Utz, J. P., Fahey, J., Frei, E., and Kalb, M., The Nature of the antibody response in acute leukemia. In preparation.

3. R. T. Silver, Beal, G., Schneiderman, M., and McCullough, N. The Role of the Mature Neutrophil in Bacterial Infections in Acute Leukemia. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

None.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NC1-4-717(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$12,350	$19,000	$31,350	1	2/3	1 2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	2/3	1 2/3	221

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-4-719(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Leukemia Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. A Study of the Clinical Manifestations of Acute Leukemia
PROJECT TITLE

7. Dr. Emil Frei, III
PRINCIPAL INVESTIGATOR

8. Dr. Claude Forkner, Dr. Richard Fritz, and Dr. James Stabenau
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To delineate etiologic and pathogenetic factors of the various manifestations of acute leukemia with particular emphasis on the oral, neurologic, hepatic, renal, and osseous lesions and the abnormal uric acid metabolism.

Methods Employed: The comprehensive application and correlation of clinical, hematological, radiologic, chemical, microbiologic, and histologic methods to the above manifestations in patients with acute leukemia.

(Use reverse and additional pages, if necessary)

Major Findings: 1. Oral Lesions - These are a very frequent manifestation of acute leukemia and occur as a toxic manifestation of folic acid antagonists. There is evidence from tissue culture studies that folic acid antagonists arrest mitosis in the mitaphase. In an effort to delineate etiologic and pathogenetic factors in the production of oral lesions a consecutive series of such lesions were studied clinically, microbiologically and histopathologically (biopsy). In the 6 biopsies taken from lesions thought clinically to represent methotrexate toxicity no nuclear or mitotic abnormalities were found.

2. Renal Lesions: A retrospective study of 62 consecutive cases of acute leukemia revealed the following:

A. Acute renal failure associated with, and perhaps due to, hyperuricemia following antileukemic therapy. (5 cases)

B. Uremia secondary to nephrocalcinosis in patients with extensive bone disease. (2 cases)

C. Uremia secondary to pyelonephritis. In one of these necrotizing papillitis occurred. (7 cases)

D. Marked enlargement (> 2x) of kidneys due to diffuse leukemic infiltration

1) with renal failure 3 cases
2) without renal failure 8 cases

E. Marked enlargement (>2x) of kidneys in the absence of gross or microscopic abnormalities. Is the hyperplasia compensation for the increased metabolic load? Liver hyperplasia is known to occur in leukemia. (3 cases)

3. Hepatic Lesions: This study was prompted by the observation that jaundice occurs frequently in acute leukemia and that pathologic changes are frequently noted in the liver at autopsy. In this retrospective study jaundice (bilirubin > 2 mg.%) occurred 23 times in 52 consecutive patients. In general jaundice tended to occur when the patient was otherwise severely ill. As several possible etiologic factors were usually present it was difficult to be certain of etiology in any given case. Twelve of the episodes of jaundice occurred in association with bacteremias. Nine of these twelve episodes of bacteremia were due to gram negative rod infections. Combined 6-MP

Major Findings (cont'd)

and Mtx toxicity was present within 10 days of the onset of jaundice in 8 cases. In one of these the subsequent administration of 6-MP again produced jaundice. One episode was clearly the result of the administration of incompatible blood. One patient had jaundice as the presenting symptom of her leukemia. She had giant hepatomegaly which with the jaundice persisted throughout her disease. Viral hepatitis, infectious or serum, may well have been responsible for several of the episodes.

The pathology analysis is not yet complete. One case of antimetabolite jaundice died during the episode. Nonspecific patchy necrosis was noted in the liver. The amount of leukemic infiltrate varied greatly. In only one case (above) could leukemia alone be considered the cause of jaundice. In this case very extensive diffuse leukemic infiltration of the liver was noted at autopsy. Otherwise the occurrence of jaundice did not correlate with the magnitude of liver infiltrate. Other pathologic changes noted were fatty infiltration, metastatic abscess and gangrene. (Clostridium perfringens)

4. Neurologic Lesions: In cooperation with the NINDB and the Pathology Department a comprehensive clinical-pathologic study of the nervous system in patients with acute leukemia is underway. The following are the major findings. Neurological manifestations are common, 22 of 42 cases manifesting changes at some time during the course of their disease. They result from complications of leukemia (infection and hemorrhage) or direct infiltration by leukemic cells. The most common manifestation of the latter are diffuse meningeal and peripheral nerve involvement. Neurological manifestations, particularly meningeal involvement have been observed to improve on chemotherapy in the absence of hematologic improvement and also to occur when patients are in systemic remission and on maintenance therapy. Correlation between clinical localization of the lesion and postmortem findings is poor.

The possible causal relationship between leukemic CNS localization and blood-brain distribution of antimetabolites is being investigated in cooperation with the Laboratory of Clinical Pharmacology.

Proposed Course of Project: This may be determined when the pathologic data are completed and collated with the clinical. The clinical evaluation of any given case of jaundice is difficult and often, if not usually,

Proposed Course of Project (cont'd)

inconclusive. Laboratory studies help in determining the extent of hepatocellular damage but were of no differential value except in a few cases of septicemia where in the early phases of jaundice hemolysis was prominent and later hepatocellular damage.

Careful clinical and laboratory evaluation of liver function and jaundice will continue to be made.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-4-719(C)
SERIAL NUMBER

12. BUDGET DATA:

FY'57

ESTIMATED OBLIGATIONS			MAN YEARS		
DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
$13,330	$24,870	$38,200	1	2/3	1 2/3

FY'57

BUDGETED POSITIONS			PATIENT DAYS
PROF	OTHER	TOTAL	
1	2/3	1 2/3	300

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards and Publications 15. NCI-4-719(C)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Gold, L. G., and Fritz, R. Hyperuricemia Associated with the Treatment of Acute Leukemia. Submitted to Annals of Internal Medicine.

Wells, C., and Silver, R. T. The Neurological Manifestations of the Acute Leukemias; A Clinical Study. Accepted for publication in the Annals of Internal Medicine.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-720(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Metabolism Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Amino Acid Metabolism in Man
PROJECT TITLE

7. John L. Fahey, M. D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The general objective is to study the human metabolism of amino acids in normal and disease states. Specific objectives are (a) to establish the conditions by which amino acids are most effectively utilized when administered intravenously, and (b) to develop an understanding of amino acid - ammonia relationships in man.

Methods Employed: L-amino acid mixtures in aqueous solution have been administered intravenously. Serial blood and urine samples were obtained and, on appropriate samples, ammonia, urea and alpha amino nitrogen determinations have been carried out. The clinical response of the subjects was also observed.

(Use reverse and additional pages, if necessary)

Patient Material:

	Number	Total Stay(days)
Admissions	9	333

Major Findings:

1. A marked blood ammonia rise following intravenous administration of an arginine deficient amino acid mixture and the effectiveness of L-arginine in preventing the ammonia rise, demonstrated in dog studies last year, has been confirmed in both respects in man.

2. Further studies have demonstrated that L-citrulline and L-ornithine are also effective in preventing toxicity and the blood ammonia rise. D-arginine and L-glutamic acid were ineffectual.

3. Observation on the rates of urea formation indicate that arginine is active through its role in the Krebs-Henseleit ornithine-urea cycle.

4. Glycine administration will cause an ammonia rise similar to that produced by the arginine-deficient L-amino acid mixture.

Proposed Course of the Project: (A) Observations will be conducted to determine the site(s) of ammonia formation following amino acid administration, and the site of arginine activity. Comparison will be made of the ammonia-forming potential of individual amino acids administered intravenously.

(B) Assessment of the activity of L-arginine in reducing elevated blood ammonia levels associated with ammonium infusions and with hepatic disease will be undertaken.

Significance to Cancer Research: Further information on amino acid disposition and nitrogen transfer within the intact subject will provide a better base from which to investigate tumor metabolism and the metabolism of the tumor-containing host. Clinically, development of means of managing the toxic effects of elevated blood ammonia levels would be important in the management of hepatic damage states and in conditions of alkalosis in which ammonium chloride administration is indicated. Safe, profitable intravenous administration of amino acids is important in the general medical care of many patients with neoplasm.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-720(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$75,680	$34,280	$49,960	1	1	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for items 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. CR-1
October 1956
(Attachment I)

Calendar Year 1958

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-721(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Metabolism
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Metabolism of nutrients and calories in subjects with malignant disease.
PROJECT TITLE

7. Donald M. Watkin, M.D.
PRINCIPAL INVESTIGATOR

8. Jesse L. Steinfeld, M.D. and Charles B. McCall, M.D.; in addition, Residents Mehran Goulian, M.D., Herbert Levine, M D., Bernard Landau, M.D., ~~Richard Paton, M.D., Daniel Nathans, M.D., and~~ Robert Mendelsohn, M.D.
OTHER INVESTIGATORS
Clinical Associates: David Nathan, Gurstin Goldin, Robert Weiger, Wm. Peiper, Thomas Waldmann, and Sherman Weissman.

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: Investigation of the over-all and intermediary metabolism of protein, fat, carbohydrate, minerals, electrolytes, vitamins, calories and water in the natural course of malignant disease and in the response of the disease to nutritional, chemotherapeutic, endocrine, surgical or radiologic therapy.

Methods Employed: The metabolic balance technique is used to quantify changes in composition of tumor and host by measuring differences between the intake and output of body constituents. The calculation of insensible water loss from water balance together with analysis of expired air (Dr. McCall) are used to estimate energy expenditure. Radioisotope techniques are utilized to measure albumin turnover (Dr. Steinfeld).

(Use reverse and additional pages, if necessary)

Calendar Year 1956

NCI-6-721(C)

Patient Material:

In patients, January 1, 1956:	2
In patients admitted, January 1, 1956 - December 31, 1956	
Initial Clinical Center admissions:	5
Repeat Clinical Center admissions:	7
In patients other than above studied during 1956:	6
Deaths:	1
Autopies:	1
Outpatients:	
Screening:	5
Follow-up:	4
Research:	1
Total patient days attributable to patients studied on this project:	516

Major Findings: Since three studies were conducted under the general heading of C-6-721(C), a separate description of each study is reported.

A. Nitrogen, albumin, mineral, electrolyte and energy metabolism during hyperalimentation by intravenously administered fat emulsion in subjects with malignancy and cachexia.

Objectives: Quantification of the effects of excessive calories supplied by fat administered intravenously on host and tumor; evaluation of caloric hyperalimentation as a therapeutic measure; exploration of the relationships among energy metabolism, fat, protein and carbohydrate metabolism, and tumor growth.

Methods Employed:

a) Metabolic balance technique including determination of energy expenditure by measuring insensible water loss (Newburgh).

b) Measurement of respiratory quotient (R.Q.) and basal metabolic rate (B.M.R.) by the open circuit method.

c) I^{131} labelled human serum albumin turnover.

Patient Material: In the calendar year 1956, one woman with breast carcinoma, one man with post-gastrectomy cachexia, one man with diabetes mellitus, one woman

Patient Material (cont'd): with massive distal small bowel resection and one boy with ulcerative colitis were under initial study. One man with chondrosarcoma and one with hypernephroma who received hyperalimentation in 1955 returned for follow-up examinations.

Major Findings: In the control phases of these studies, prior to hyperalimentation, patients with cancer were in negative caloric balance, were burning a metabolic mixture composed largely of fat, and were found to have R.Q.'s well below 0.75. The chronically ill but non-cancerous patients were in caloric equilibrium, were burning a metabolic mixture of mixed composition and had R.Q.'s of 0.8 or above. In response to hyperalimentation, patients with cancer increased their caloric expenditure to maintain a negative caloric balance similar to or greater than that observed in the control periods. Weight gains were associated with positive nitrogen, potassium, phosphorus and sodium balances, indicating that caloric deficits could be attributable to metabolism of large quantities of fat. Non-cancerous patients responded to hyperalimentation by positive caloric balances accompanied by weight gain and positive nitrogen, potassium, phosphorus and sodium balances. The feasibility of using intravenous fat emulsions in the presence of severe diabetes mellitus treated by insulin was demonstrated. The introduction of an emulsion containing a new stabilizer has eliminated initial reactions and febrile responses noted previously. Patients with and without cancer continue to demonstrate BSP retentions and mild anemias during fat emulsion infusions. One year follow-ups on two cancer patients revealed the BSP retention and anemia associated with the infusions to be transient phenomena. Patients with and without cancer showed an increase in total albumin and the rate of albumin turnover during hyperalimentation.

Significance to the Program of the Institute: These studies demonstrate definite differences between patients with cancer and those suffering from non-cancerous chronic disease in caloric expenditure, in composition of the metabolic mixture, in respiratory quotient and in response to hyperalimentation. A generalized metabolic abnormality associated with, but not necessarily caused by, the presence of tumor tissue is indicated. The practicality of fat emulsions as a means of supplying caloric hyperalimentation is established.

Proposed Course of Project: Direct measurements of the rate of fat metabolism using emulsions containing I^{131} labelled triolein in patients with and without cancer before and during caloric hyperalimentation. Confirmation of reported findings by improved methods of indirect calorimetry. Investigation of possible techniques for preventing observed abnormalities in energy metabolism in patients with cancer, before and during caloric hyperalimentation.

B. Metabolic and clinical effects of massive doses of prednisone in subjects with malignant disease (also listed as C-6-701A(C)).

Objectives: To quantify the effects of massive adrenal corticoid therapy on nitrogen, mineral, electrolyte, water and energy metabolism in patients with malignant disease.

Methods Employed: (a) Metabolic balance technique including determination of energy expenditure by measuring insensible water loss (Newburgh). (b) Measurement of respiratory quotient (R.Q.) and basal metabolic rate (BMR) by the open circuit method. (c) I^{131} human serum albumin turnover. (d) Discrete renal function studies. (e) Electrophoretic analysis of serum protein (performed by Dr. J. L. Fahey).

Patient Material: Two women, one with multiple myeloma and one with lymphosarcoma, and four men, one with Hodgkins' Disease, one with multiple myeloma, and two with prostatic carcinoma. With the exception of one patient with prostatic carcinoma, these individuals were in-patients during calendar 1955. However, all computations on which interpretations are based were performed in calendar 1956. Hence these studies are being reported here for the first time.

Major Findings: Prednisone therapy was accompanied by a decrease in the calculated fat of the metabolic mixture and a decrease in caloric expenditure calculated by the Newburgh technique. Nitrogen, potassium, and phosphorus balances were markedly negative. Actual phosphorus balances were greater than those theoretically attributed to nitrogen and calcium balances. Sodium and chloride retention occurred. Uric acid excretion was slightly elevated in three and markedly elevated in two patients. Marked negative phosphorus and nitrogen balances and markedly elevated uric acid

Major Findings (cont'd): excretions were associated with objective diminution in tumor size in patients with lymphoma and Hodgkins' Disease. Albumin turnover was accelerated but albumin synthesis depressed during prednisone administration confirming the "antianabolic" quality of cortico steroids. Renal plasma flow was reduced markedly, apparently due to reduction in cardiac output leading in one patient to definite forward failure. Electrophoretic studies of plasma proteins revealed for the first time a change in the characteristic pattern in a patient with multiple myeloma. Clinical complications of massive prednisone therapy included acute psychosis, systemic moniliasis, congestive heart failure, diminished renal plasma flow, pulmonary embolism, paroxysmal tachycardia, pneumonia, severe acne, thrombocytopenia and severe arthralgia on withdrawal. Administration of small doses of prednisone to a patient with prostatic cancer who demonstrated an extremely low R.Q. resulted in an abrupt elevation of R.Q. to normal with a gradual escape to control values. This response to prednisone parallels similar findings in another patient studied before and after by hypophysectomy and suggests that prednisone suppresses a pituitary factor responsible for the low R.Q. and the high proportion of fat in the metabolic mixture.

Significance to the Program of the Institute: Prednisone offers a tool by which a generalized metabolic abnormality observed in patients malignancy can be shifted temporarily at least in the direction of normal. It has therefore considerable utility in pursuing the problems of energy metabolism in cancer.

Proposed Course: The ability of prednisone to suppress the pituitary factor responsible for lowering the R.Q. will be checked in patients destined eventually for hypophysectomy. Further examination of the reason for nitrogen retention in excess of theoretical based on phosphorus balance is indicated. The "antianabolic" action of prednisone as measured by its effect on serum albumin synthesis will be pursued.

C. The respiratory quotient (R.Q.) and basal metabolic rate (B.M.R.) in patients with cancer, with non-cancerous chronic disease, and in normal controls.

Objectives: Demonstration of an abnormal metabolic pathway in patients with cancer. Investigation of possible factors responsible for the abnormality.

Calendar Year 1956

NCI-6-721(C)

Objectives (cont'd): Correlation with other energy metabolism studies.

Methods Employed: Quantitative collection of expired air and its analysis for CO_2 and O_2.

Patient Material: Three normal controls. Three patients with non-malignant chronic disease. Nine patients with malignant disease.

Major Findings: Nine patients with malignant disease have demonstrated R.Q.'s averaging .74 with a range of 0.66 to 0.82. Three normal controls had R.Q.'s averaging 0.79 with a range of 0.76 to 0.83. Two patients with non-malignant chronic disease had R.Q.'s averaging 0.77 and ranging from 0.71 to 0.82. Two patients with cancer were studied ten days post-hypophysectomy and both had R.Q.'s above 0.9. One diabetic in acidosis had R.Q.'s averaging 0.70. These figures, although including all determinations regardless of patient training, indicate that cancer patients on the average have R.Q.'s below those of normals and those with chronic diseases other than cancer. Suppression or ablation of the pituitary in patients with cancer was accompanied by a dramatic elevation in R.Q. Administration of 50 mg. of monkey growth hormone to one hypophysectomized patient over a three day period did not alter the R.Q. despite marked retention of nitrogen.

Significance to the Program of the Institute: The R.Q.'s reflect differences in metabolic mixture burned by patients with and without cancer and suggest a metabolic abnormality associated with cancer which can be altered by medical suppression or surgical ablation of the pituitary gland.

Proposed Course of Project: Confirmation of reported findings by improved methods of indirect calorimetry. Further investigation of the role of the pituitary in governing composition of the metabolic mixture. Inspection of the relationship between the metabolic abnormality and the presence of an anatomical tumor, i.e. is the metabolic abnormality dependent on the presence of tumor tissue.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-721(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$14,780	$44,340	$59,120	5/6	2/3	1 1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	5/6	2/3	1 1/2	516

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NCI-6-721(C)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Watkin, D. M. Controlled metabolic studies of caloric hyperalimentation with fat emulsion given intravenously in four patients with cancer and one with non-cancerous chronic disease. Proc. of the Surgeon General's Task Force for Intravenous Fat Alimentation, Denver, Colorado, October 4-5, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. CRP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-721D(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Nutrition and Metabolism Service
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Characterization of over-all Metabolism in Chronic and Acute Leukemia and the changes induced by antileukemic therapy.
PROJECT TITLE

7. Donald M. Watkin, M. D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

This project has been incorporated under other headings: Nutrient and energy metabolism in subjects with malignant disease, NCI-6-721A(C), NCI-6-721C(C), NCI-6-725(C), and NCI-6-726(C).

Brief Summary: One patient with chronic myelocytic leukemia has been followed weekly during out-patient therapy of his disease with Colcemide. B_{12} plasma levels have been followed. Colcemide has proven effective at dose levels of 10 mg. daily and has to date been entirely non-toxic. Compared to Myeleran, with which the patient has been treated on two previous occasions, this drug at the dose level tested has produced less dramatic responses.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-721D(C)
SERIAL NUMBER

12. BUDGET DATA:

FY'57

ESTIMATED OBLIGATIONS			MAN YEARS		
DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
$15,090	$9,500	$24,590	1	1 1/3	2 1/3

FY'57

BUDGETED POSITIONS			PATIENT DAYS
PROF	OTHER	TOTAL	
1	1 1/3	2 1/3	110

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entry for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-722(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Metabolism
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Measure of blood volume, red cell mass and total circulating albumin in patients with cancer.
PROJECT TITLE

7. J. L. Steinfeld, M.D.
PRINCIPAL INVESTIGATOR

8. ---
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

This project is completed. The final findings are in accord with those presented in 1955 Annual Project.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-722(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,960	$1,570	$5,530	1/3	1/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-723A(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Metabolism
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Distribution and degradation of human serum albumin labelled with I^{131} by different techniques.
PROJECT TITLE

7. Jesse L. Steinfeld, M.D.
PRINCIPAL INVESTIGATOR

8. R. E. Paton, M.D., Arnold Flick, M.D., Robt. Milch, M.D., F. E. Beach and Dr. Donalee L. Tabern (Abbott Laboratories)
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To ascertain if different techniques of labelling albumin with I^{131} alter to a different degree the biologic behavior of the I^{131} albumin.

Methods Employed: Chromatography, dialysis, TCA precipitation - then administration in trace quantities 15 - 20 μc I^{131} to patients with collection of blood and urine specimens for assay of radioactivity.

Patient Material: Twenty-two (22) patients already on the wards of the National Cancer Institute for other studies or therapy participated in this research program. Two (2) patients were admitted for 23 days to serve as patients for this study alone.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

NCI-6-723A(C)

Major Findings: The I^{131} albumin studied gave comparable values for blood volumes and total exchangeable albumin despite a 20 fold difference in self radiation.

The use of hypochlorite-iodide instead of iodine as the iodinating agent increases the rapidity with which I^{131} tag is lost from the protein.

An increase in self radiation in rep from 10,000 to 40,000 to 200,000 to albumin in a concentration of 10 mg/cc increasingly alters the albumin so that it is more rapidly lost from the body even as late as the tenth day.

Significance to Cancer Research: Studies on albumin metabolism in cancer patients with I^{131} albumins have been made on the assumption that all I^{131} albumins were homogeneous with respect to each other. It may be possible to re-examine earlier studies now in view of above findings and determine which are reasonably free of criticism and which are not.

Proposed Course of Project: Completed.

Form No. ORP-1
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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-723A(C)
SERIAL NUMBER

12. BUDGET DATA:

ESTIMATED OBLIGATIONS			MAN YEARS		
DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
$3,960	$1,570	$5,530	1/3	1/3	2/3

FY'57

BUDGETED POSITIONS			PATIENT DAYS
PROF	OTHER	TOTAL	
1/3	1/3	2/3	0

FY'57

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-723B(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Metabolism
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Distribution and degradation of I^{131} albumin and I^{131} polyvinyl pyrollidone in normal subjects and patients with regional enteritis or ulcerative colitis.
PROJECT TITLE

7. J. L. Steinfeld, M D., R. S. Gordon, Jr., and J. D. Davidson, M.D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To study the hyperalbuminemia in patients with ulcerative gastrointestinal disease and to develop a diagnostic test for patients suspected of having ulcerative gastrointestinal disease.

Methods Employed: I^{131} labelled albumin or PVP is given IV or p.o. to individuals receiving Lugol's Solution. Twenty-four (24) hour urine and stool collections are assayed for free and bound radioactivity for from 7 to 28 days.

Patient Material: Of 8 subjects hospitalized on the NHI Service, 4 with gastrointestinal disease and 4 without have been studied to date.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

NCI-6-723B(C)

Major Findings: An increased rate of I^{131} albumin degradation comparable to that seen in patients with hyperthyroidism is found in 3 patients with regional enteritis.

Patients with diarrhea have 10 - 100 times as much I^{131} as iodide in their stools as do patients without diarrhea. This is not specific for patients with gastrointestinal disease since when diarrhea was induced in three subjects without gastrointestinal disease, the iodide partition between stool and urine was similar to that of the patients with ulcerative gastrointestinal disease and diarrhea.

Significance to Cancer Research: The fact that patients with ulcerative gastrointestinal disease have hypoalbuminemia and an increased turnover rate of albumin suggests that the albumin is broken down in the lumen of gastrointestinal tract by the digestive enzymes there. (Immunologic study reveals no intact albumin to be in the stool.) Such degradation of albumin may or may not suggest that normal albumin breakdown occurs in the lumen of the gastrointestinal tract although this concept remains to be investigated.

Proposed Course: To collect data on more patients with regional enteritis and ulcerative colitis. To check if I^{131} PVP given p.o. enters the blood stream through an ulcerated, but not a normal gastrointestinal tract and vice versa - this is to be used possibly as a diagnostic test for extensive ulcerative gastrointestinal disease.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-723B(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,960	$1,570	$5,530	0	2/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	2/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-724(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Metabolism
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Simultaneous measurement of total exchangeable sodium and potassium in cancer patients.
PROJECT TITLE

7. J. L. Steinfeld, M.D. and R. E. Paton, M.D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: (1) To develop methods for the simultaneous determination of total exchangeable sodium and potassium.

(2) To study in some detail electrolyte disturbances in cancer patients ((a) hyperchloremic acidosis of patients with metero-sigmoidostomies, (b) patients with hypophysectomies, aldosteromas, etc.).

Methods Employed: Use of different biologic fluids (urine serum saliva) and different counting systems (gas G-M and scintillation) will permit for the first time the simultaneous measurement of these isotopes when given in tracer quantities.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

NCI-6-724(C)

Methods Employed (cont'd): The measurements - both of Na^{24}, Na^{23}, K^{42} and K^{39} will be made 18 - 24 hours after IV administration of 1.5 μc/Kg of each isotope.

Patient Material: Nine (9) patients on the NCI wards have been studied to date.

Major Findings: As was believed on a theoretical basis, it is possible to simultaneously measure Na^{24} and K^{42} in the same or different biologic fluids.

No data are yet available on electrolyte abnormalities of specific clinical entities.

Significance to Cancer Research: It will be possible to study in greater detail and with greater accuracy some of the electrolyte disturbances which are frequently seen in patients with advanced cancers.

Proposed Course of Project: As outlined under objectives 1 and 2.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-724(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$5,940	$2,370	$8,310	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entires for items 15, 16 and 17.

(Use reverse and additional pages, if necessary)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-725(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Nutrition & Metabolism Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Vitamin B_{12} metabolism in normal subjects and in subjects with malignant disease.
PROJECT TITLE

7. Donald M. Watkin, M.D.
PRINCIPAL INVESTIGATOR

8. Robert S. Mendelsohn, M.D.
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: Quantitative observations on the metabolism of vitamin B_{12} in man, its level and site of binding in blood plasma, its absorption and distribution in the bodies of normal subjects and those with malignant disease, and its excretion by the kidney.

Methods Employed: Microbiological assay (L. leichmannii) for vitamin B_{12} in plasma, urine and tissue. Measurement of Co^{60} labelled radioactive B_{12} in blood, urine, tissue and feces. Column fractionation of plasma protein. Renal clearance techniques.

Patient Material: The renal clearances of B_{12} at plasma levels rising from endogenous to 70 mugm. per ml. were measured in four normal subjects.

(Use reverse and additional pages, if necessary)

Patient Material (cont'd)

B_{12} absorption after oral ingestion has been measured using the Schilling test in 20 young and 20 old normal subjects. Serum B_{12} levels have been determined by microbiological assay in 45 subjects with malignant disease. Fractionation of serum proteins by column chromatography and the assay of the fractions for B_{12} activity has been performed in the sera of two normals and one subject with chronic myelogenous leukemia.

Major Findings: In three out of four normal subjects, the urinary excretion of B_{12} per ml. of glomerular filtrate was 15 to 30 mugm. per ml. below plasma concentration at plasma levels ranging from 50 to 70 mugm. per ml. This indicates excessive plasma B_{12} binding at high levels, relative trapping of B_{12} by kidney parenchyma, or reabsorption of filtered B_{12} associated with a constant rapid destruction, storage or extraurinary excretion of assayable vitamin.

Schilling tests have demonstrated no significant differences in absorption of orally ingested B_{12} between young and old normal subjects.

The method for assay of vitamin B_{12} by L. leichmannii in biological material has been refined and its limits of error quantitatively determined.

The vast majority of subjects with malignant disease have had serum B_{12} levels in the normal range. One subject with chronic myelocytic leukemia and three with malignant melanoma had B_{12} plasma levels far above the normal range. Fractionation of serum protein and assay of the fractions indicate that B_{12} activity both in normal serum and in the serum of a subject with chronic myelocytic leukemia is located in certain fractions included in the alpha globulins.

Significance to the Program of the Institute: Studies in normal subjects have provided essential controls for the investigation of B_{12} absorption, distribution, excretion and rate of metabolism in subjects with malignant disease. The high serum B_{12} level in chronic myelocytic leukemia and the quantitative increase in normal B_{12} binding capacity of an alpha globulin component are objective differences between the healthy subject and the subject with malignant disease which suggest an underlying metabolic defect possibly responsible for and not necessarily resulting from the malignant

Significance to the Program of the Institute (cont'd)

state. The finding of elevated serum B_{12} levels in malignant melanoma suggests a metabolic defect in common with chronic myelocytic leukemia.

Proposed Course of Project: Techniques for handling Co^{58} in addition to Co^{60} labelled radioactive vitamin B_{12} are being developed. The assay for B_{12} activity of serum from each subject with malignant disease admitted to The Clinical Center will be continued until a statistically adequate sample of each of several representative malignancies is obtained. In selected subjects with abnormal levels fractionation of the serum and assay of the fractions for B_{12} activity will be continued. The location of B_{12} binding capacity in the spectrum of serum proteins will be examined by in vitro and in vivo experiments using Co^{58} labelled B_{12} as well as the microbiological assay technique. In selected subjects, B_{12} distribution studies using renal clearance techniques will be performed. Distribution of radioactive cobalt labelled B_{12} in normal and malignant tissue will be determined in surgical and autopsy specimens.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NOI-B-7-3(C)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Gaffney, G. W., Watkin, D. M., Shock, N. W. and Chow, B. F. Urinary excretion of orally administered Co^{60} - labelled vitamin B_{12} in human adult males at various ages. J. Gerontology (In press),

(2) Watkin, D. M., Barrows, C. H., Jr., Chow, B. F., and Shock, N. W. The renal clearance of intravenously administered vitamin B_{12}. J. Gerontology (In press).

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. N[illegible]-C-25(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$5,940	$2,370	$8,310	-		

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	1	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Gerontology Branch, National Heart Institute.

(Use reverse and additional pages, if necessary)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-6-726(C)
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. General Medicine Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Metabolism
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Renal clearances in malignant disease
PROJECT TITLE

7. Donald M. Watkin, M.D.
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To examine the renal handling of metabolites such as uric acid and calcium at various plasma levels in malignant disease.

Methods Employed: Renal clearance technique.

Patient Material: One man with hyperuricemia in acute leukemia; one man with low serum uric acid in prostatic carcinoma; one woman with hyperuricemia and myeloma kidney in multiple myeloma; one man with hyperuricemia and hypercalcemia in bronchogenic carcinoma.

Major Findings: These investigations have raised to 12 the number of

(Use reverse and additional pages, if necessary)

Major Findings (cont'd): renal clearance studies performed in a total of nine patients. It is now apparent that patients with hyperuricemia may be separated by these studies into those whose hyperuricemia is due to renal disease and those whose hyperuricemia is due to over-production of uric acid. In the former group there is an inverse and in the latter a direct relation between the plasma uric acid level and the tubular transport of uric acid (T_{UA}). Since no serial studies were performed in the same individual, further observations on the previously reported reciprocal relationship between T_{UA} and TmPAH were not obtained. In one patient calcium clearance was measured using the centrifuge type of ultrafiltration apparatus for the measurement of ionized calcium in plasma. GFR, RPF and TmPAH were reduced to 25 per cent or less of normal. Despite hypercalcemia, over 90 per cent of filtered ionizable calcium was reabsorbed. Elevation of PAH plasma levels to 0.5 mg./ml. (load/Tm = 12.7) resulted in a 15 per cent reduction in reabsorption of filtered calcium. This implies a suppression of tubular calcium transport at high load/TM ratios of PAH.

Significance to the Program of the Institute: Plasma uric acid is determined in part by renal tubular reabsorption of uric acid, in part by the rate of uric acid synthesis and in part by the release of uric acid and its precursors from tissues undergoing destruction. The renal contribution to the maintenance of plasma level must be known before hyperuricemia can be ascribed to increased synthesis or to destruction of tumor tissue. Hypercalcemia is a dangerous complication of metastatic disease. It is a precursor of renal damage which in turn aggravates the hypercalcemia. Investigation of renal mechanisms for clearing calcium in hypercalcemia are basic to a solution of this problem.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-6-726(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$5,940	$2,370	$8,310	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NCI-6-726(C)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Stoloff, I., Watkin, D. M. and Shock, N. W. Age and the ratio TmPAH/Tm diodrast in man with a note on the self depression of Tm diodrast. J. Gerontology, 11:388-390, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-750-C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4.
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Removal of Cancer and Other Tissues for Histologic, Biochemical and Other Studies as Required by Scientists and Other Investigators for Correlated Studies.
PROJECT TITLE

7. Dr. Robert R. Smith.
PRINCIPAL INVESTIGATOR

8. Dr. William E. Schatten, Dr. Arthur G. Ship, Dr. Robert A. Milch, Dr. William M. Kramer, Dr. Horace Herbsman, Dr. James R. Jude, Dr. P. Olch, Dr. Chester Haverback, and Dr. Peter H. Doubler.
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

The object of this project is to supply human tissue for histologic, histochemical, biochemical and biophysical studies to scientists working in various branches of the Cancer Institute who require tissue for studies. As a rule, such studies are done as cooperative projects with various investigators but on occasions specific types of tissue are required for specific studies. For example, one investigator requested that specimens of skin and its associated neoplasm be made available for keratinization studies, and various muscle biopsies and/or biopsies of liver tissue under

(Use reverse and additional pages, if necessary)

C-10-750-C
SERIAL NUMBER

Objectives: (cont'd)

specific conditions have been requested. These materials are obtained during operations for standard therapeutic reasons.

Methods Employed:

Methods employed are standard surgical procedures.

Patient Material:

Material for this project is obtained mostly from patients hospitalized for study in other projects. Occasionally, however, it is necessary to admit patients for specific studies in which a specific type of tissue is obtained. There have been several examples of this, mostly at the request of the dermatologist who wanted large volumes of basal cell carcinomas for a specific study. However, even in these studies, data for other projects were obtained, for example, the wound seeding study. On several occasions patients were admitted by other institutes for material for special *in vitro* studies, (mostly thyroid adenomas), and patients with obstructive jaundice in order to obtain bile for biochemical studies. It is difficult from the data available to determine just which cases were admitted for specific studies. However, some 130 minor operative procedures were done for patients admitted on other services for biopsies and/or biochemical, biophysical, and histochemical studies.

Major Findings:

Presented in reports of other projects by other investigators.

Significance to Cancer Research:

This project is more or less a service function for other branches of N. C. I. and N. I. H.

Proposed Course of Project:

The number of such cases will continue at about the present level in proportion to the patient load.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-750-C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$6,220	$10,050	$16,270	1	0	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	0	1	117

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-751-C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4.
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Evaluation of Radical Surgery and the Development of New Surgical Techniques as a Therapeutic Means of Palliating or Definitive Therapy of Advanced Cancer.
PROJECT TITLE

7. Dr. Robert R. Smith
PRINCIPAL INVESTIGATOR

8. Dr. John H. Waite and Dr. William E. Schatten
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

The objectives of this project are to develop methods of obtaining increased palliation and/or definitive cure of advanced cancer. Clinical material available for this type of study is largely limited to cancers of the head and neck area and carcinomas of the female pelvis.

Methods Employed:

Patients with advanced cancer of the pelvis and/or head and neck areas are admitted for study. These patients have locally operable but advanced disease without evidence of spread beyond the operative area.

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

DESCRIPTION (Continued)

Methods Employed: (cont'd)

The project is in reality composed of a number of projects of specific sites, but because of the relative infrequency of cancer of specific areas, studies must be arranged to take advantage of the clinical material as it becomes available. In previous reports, studies of paranasal sinus cancer has been discussed. During the past year, one or two additional patients in this category have been seen and studied. This type of surgery, done in conjunction with the neurosurgeons, consists of widening the field of the excisional surgery. Carcinoma of the female pelvis, mostly cervix cancer, has been more readily available, and in conjunction with the cervix-virus study project has supplied considerable material for this project. The radical approach to advanced cervix cancer has consisted of developing the techniques of exenterative surgery. More recently, emphasis has been placed upon the development of a technique for managing the urinary stream after excision of the urinary bladder. In other head and neck cancers the emphasis has been placed upon the development of adequate excisional therapy in conjunction with the plastic repair, allowing cosmetic and functional results.

Patient Material:

As noted in previous reports, there is considerable overlap of patient material. The number of patients admitted specifically for this study is given below.

Major Findings:

Analysis of the data of radical surgery for cervix cancer is not complete at this time. The first patient on whom a pelvic exenteration was performed died about two years postoperatively with extensive lung and supraclavicular metastases. It becomes apparent that as the local disease is adequately controlled and the surgery is successful in controlling the diseased area of the primary and its lymph node drainage area, the systemic manifestations of the disease develop more frequently. It is noted, for example, that in the first 28 patients operated upon in this group, 11 are known to be dead at the present time. Of the 20 cases that have been autopsied, 7 had liver metastases, 8 had lung metastases, 3 had bone metastases, and 14 had lymph node metastases outside of the pelvis. A more complete analysis of this group of cases is now underway.

At the present time it is possible to note the difference in the causes of death in this group as compared with the usual autopsied series as reported in the literature. In such series, uremia is a very frequent cause of death; for example, in the Pondville series,

Major Findings: (cont'd)

ureteral obstruction was noted in 70% of their cases and was severe enough in 40% to lead to uremia. 22% died of peritonitis in their series, none of which was noted in this hospital series. In the Pondville group it was noted that the great majority of patients dying of their cervical carcinoma did not have metastases outside the pelvis. In only 23% of their 169 cases were distant metastases found. It was noted that in all of the cervix cancer cases autopsied here, with the exception of one or two cases in which no distant metastases were identified, the patients died with widespread dissemination of their cancer.

The radical paranasal sinus surgery continues to give good results. Several new cases were operated upon and found inoperable. One case was admitted with an ethmoid cancer and was found operable and has had a good immediate result. The problem with this type of cancer is the difficulty in finding an adequate number of cases to study. Another complication that requires study to correct is the extensive crusting which develops in the region of the skin graft. A mucous-secreting graft would be ideal if available.

Significance to Cancer Research:

We believe that the development of effective means of palliating and/or curing advanced cancer is one of the fundamental purposes of the Cancer Institute's program. Documentation of the course of cancer of these areas as the patients continue through the course of their disease adds considerably to the total knowledge of the natural behavior of cancer. It should also be pointed out that this project provides considerable material for both excised adjacent normal tissues as well as the cancer itself for scientists to use in other projects in the Cancer Institute.

Proposed Course of Project:

During the coming year it is proposed to continue the studies as outlined above. It is anticipated, now that a fairly useful and more easily adaptable type of urinary bladder diversion is available, that more exenterative surgery may be attempted. The diversion of the urinary stream from the fecal stream has resulted in partial elimination of the problem of ascending urinary infection. The problem encountered now is one in providing adequate drainage so that the obstructive symptoms do not lead to the development of ascending infection.

It is hoped that additional cases of advanced but locally operable paranasal sinus cancer will be obtained in order to further study the neurophysiological changes involved in this type of surgery.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-751-C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$44,190	$225,810	$270,000	5	0	5

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	5	0	5	2624

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16 and 17.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-[illegible]-752-C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Clinical Investigation in the Use of Viruses in the Treatment of Human Cancer.
PROJECT TITLE

7. Dr. Robert R. Smith and Dr. Robert J. Huebner
PRINCIPAL INVESTIGATOR

8. Dr. W. P. Rowe, Dr. R. G. Suskind and Dr. R. Love.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

To develop a chemotherapeutic treatment for carcinoma of the cervix, using tissue culture fluid containing live virus, attempting to reproduce the observations noted in vivo, that is, the oncolytic effect of viruses on cancer tissue growing in tissue culture.

Methods Employed:

Human cervix cancer patients were treated by the injection of tissue culture fluid containing live viruses by direct injection into the cancer, by intra-arterial and intravenous injection into the patient.

(Use reverse and additional pages, if necessary)

In addition, a number of cases were treated at the time of exploratory laparotomy for excisional surgical therapy by direct injection into the cancer through the open pelvis. The viruses used have been adenoidal-pharyngeal-conjunctival (APC) viruses grown in the virus laboratory of the Laboratory of Infectious Diseases. The viruses were grown in 5% chick serum in Hanks-Simms solution containing HeLa cells. After adequate tests for potency and safety, the fluids were injected into the patients. After injection of the virus into a number of patients, it became apparent that the antibody response in the patient promptly prevented the continued action of the virus. In an effort to overcome this and to delay or possibly prevent the production of antibodies, a number of patients received rather large doses of cortisone.

Patient Material:

Throughout the study, 31 patients with epidermoid carcinoma of the cervix were treated in this manner. 9 had clinical evidence of widely disseminated cancer, 17 had extensive pelvic tumors without clinical evidence of spread above the pelvis, and the remaining 5 had large cervical tumors limited to the cervix and adjacent parametria. In 11 of the 31 patients in this group, definitive surgical therapy was possible. Six total pelvic exenterations and 5 radical hysterectomies and lymph node dissections were performed on these patients. In a number of instances, patients received multiple inoculations, allowing a total of 48 observations.

Major Findings:

The result of the injections of the APC virus suspensions was a production of an area of necrosis and cavity formation in the central portion of the pelvic tumor. This was usually noted on the 4th to 6th day post-inoculation, but in some cases was delayed as late as the 10th day. The effect was limited to the cancer tissue, and no evidence of change in the vaginal, rectal, or bladder mucosa or other tissues of the pelvis was noted. No evidence of a systemic infection, such as one would expect to see in the natural occurring adeno-pharyngeal infection, was observed. The virus inoculations did not produce any harmful effects. In three instances systemic manifestations of a virus disease were noted. In these three patients cortisone had been given. The febrile illness was characterized by an "influenza-like" syndrome lasting 48 to 72 hours, and subsided spontaneously. In patients receiving cortisone, a more intense and prolonged effect was noted, although there was no change in the development of antibodies in these patients. In six patients, virus-free inoculum produced no observable change in the cancer. In another instance, heat-inactivated virus produced no change. In studying the effect of antibodies on the development of oncolytic effects in general it was noted that patients whose sera contained no antibodies developed a more intense local response. In all but one instance

patients whose sera contained antibodies to the virus used did not develop any oncolytic effect. Patients whose sera contained a trace of antibody responded with a 1 or 2 plus response. No maximum response was noted in this group. A maximum response was noted in 11 of the 40 instances in which it was possible to measure the response. In 14 of the 40, no local response was noted. Virus was recovered from biopsy tissue and/or vaginal smears 77 times in 20 patients. In 10 patients, 140 tests failed to recover a virus. No virus other than the one injected was ever recovered from the tumor tissue or smears except on several occasions when herpes virus was identified. The only positive virus identification from autopsy tissue was in one instance 16 days after inoculation.

The histologic changes observed in the tumor tissues following virus injection consisted of an increase in the amount of acute necrosis of the carcinoma and stromal tissue. This was often associated with acute thrombosis of many small vessels, increased edema, and inflammatory infiltrate. The pathologist noted that there was no qualitative difference from the changes usually seen in biopsy material from ulcerative cervical cancer. Independent pathologic examinations showed that the amount of necrosis of tumor and stroma noted in the biopsies, surgical specimens, and autopsies was markedly increased after virus inoculation in six instances. In another six cases the changes were considered moderate, and in three, slight. In 14 patients no quantitative change was noted in the amount of necrosis after virus inoculation. The pathological study confirms the clinical observation that in a number of cases there was increased necrosis, ulceration and sloughing of the cervix cancer. In some cases this process continued until large excavated cavities developed within the pelvic cancer. At the time of the last tabulation of these results there had been 19 deaths in this group of patients. All deaths were due to cancer, and no clinical or pathological evidence of a systemic virus disease was noted. The average time between inoculation and death was 5.4 months. In all cases extensive spread of cancer was found, and death was attributed by the pathologist to the effects of the cancer. In several instances in which the patients died during the continuing period of virus activity, extensive liquefying necrosis was extending well up into the central portion of the pelvic tumor, but this was always surrounded by a rim of viable tumor. There was no evidence found of any lesions in the central nervous system, liver or other organs which could be attributed to the injection of the virus. In no instance did the virus completely destroy the patient's cancer.

In more recent months Drs. Huebner, Suskind and Rowe in the Laboratory of Infectious Diseases have developed a laboratory model consisting of HeLa cells grown in the peritoneal cavity of x-rayed cortisonized rats. Using this model and Coxsackie viruses,

complete destruction of the transplanted cervix cancer has been obtained. This occurred as early as the fifth day following inoculation of Coxsackie B_3 virus. This oncolytic effect has been potentiated and selected by serial passage through the rat HeLa tumors. The oncolytic effect was not observed until the fifth transplant. At the present time, studies are underway using this rat HeLa oncolytic B_3 Coxsackie virus in human cervix cancer. No findings of note are available to report at the present time.

Significance to Cancer Research:

This program helps to carry out one of the fundamental aims of the Cancer Institute, that is, of developing new and more effective methods of therapy of the common malignant neoplasms. It also helps to identify and categorize many of the newly identified, little understood viruses. If a method could be developed which would allow the propagation of viruses in human tumors in situ, it is reasonable to expect that the continued propagation, not only locally but in disseminated disease, might be developed into an effective means of controlling cancer.

Proposed Course of Project:

During the coming year it is hoped that this project can be continued along several lines. At the present time the importance of demonstrating whether or not the laboratory model, that is, the destructing effect of Coxsackie viruses on the rat HeLa tumor, can be reproduced in the human patient. If by repeating in human cancer the rat HeLa experiences with Coxsackie viruses, i.e., increasing its oncolytic property, it is possible that an effective chemotherapeutic agent could be developed. The use of APC viruses will also be continued. It is hoped that in concentrating the virus particles to 500 times that present in the inoculum now used, an overwhelming infection in the tumor might be produced and in that way accomplish complete tumor destruction.

The third possible approach which lies ahead is the development of means of controlling or diminishing the host factor which would allow continued propagation of the virus in cancer cells, thus destroying all of the cancer. It is planned to attempt to lower host resistance to APC virus by making patients vitamin deficient and possibly altering their antibody response to tumor virus inoculation. It is felt that this work has demonstrated the possibility of in situ destruction of cancer by the parasitization of cancer tissue by an innocuous virus. It is felt that this approach, as an augment to the laboratory studies underway in relation to virus carcinogenesis as well as virus therapy, is an important part of the NCI program.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-9-752-C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,600	$4,620	$16,220	2	1	3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2	1	3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

Part C: Honors, Awards & Publications

15. C-9-752-C
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Paper entitled "Studies on the Use of Viruses in the Treatment of Carcinoma of the Cervix" by Robert R. Smith, Robert J. Huebner, Wallace P. Rowe, William E. Schatten and Louis B. Thomas.

Published in November-December 1956 issue of CANCER.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-S-753-C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Cytologic Determination of Number of Tumor Cells and/or Recurrences Recovered or Found in an Operative Wound Following Removal of a Primary Cancer in Continuity with its Regional Lymph Drainage Area Containing Metastases
PROJECT TITLE

7. Dr. Robert R. Smith
PRINCIPAL INVESTIGATOR

8. Dr. Albert W. Hilberg, Dr. William E. Schatten and Dr. Louis B. Thomas
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

The project is designed to attempt to demonstrate the frequency of cytologically determined cancer cells found in washings taken from major cancer surgery; correlating this information with the history, the clinical course, the operative findings, the histologic characteristics, and the results of the cancer therapy.

Methods Employed:

Patients with locally advanced primary cancers of the head and neck, pelvis, or other appropriate sites, with regional lymph node metastases,

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

Methods Employed: (cont'd)

or suspected metastases, have been admitted for primary surgical therapy. For the most part, these cases have exhibited extensive metastases, i.e., borderline operable cases. The surgery performed is the standard operative procedure accepted as treatment of choice for the specific cancer site. In part, these same patients provide material for Project C-751-C in which the limits of operability have been further defined by a more extensive resection with and without primary reconstruction.

Following removal of the tumor and its metastases, the operative area is thoroughly washed with a fine saline spray. The aspirate is fixed in an alcohol-ether mixture and sent to the cytology laboratory where additional studies are underway making it possible to identify tumor cells, individual or in clumps, from various types of body fluids.

Patient Material:

Patient material for this project was supplied by patients with primary operable but advanced cancers of various sites. Experience with the study was also obtained by necessary surgery on patients admitted for other projects. To date, approximately 125 operative procedures, with wound washings, have been performed. In an additional 42 operative procedures, washings were obtained for control studies, that is, testing the accuracy of the positive or negative reports.

Major Findings:

To date, about 125 surgical resections with wound washings will have been completed by the end of this year. Preliminary study of this group of patients shows that 27% have positive wound washings, 14% have suspicious washings, and 66% have negative washings. In control washings, false positive washings were obtained in one instance, and in an additional 3 instances washings were reported as suspicious from surgical procedures done for benign conditions. In these 4 instances (all breast surgery), the primary pathology was reported histologically as a benign lesion but containing hyperplastic epithelium. The one false positive is believed by the cytologist to be a carcinoma, but clinical histologic proof is still lacking. There were 22 instances of true positives, that is, cases in which cancer was definitely present and the cytology demonstrated the tumor cells. There were 3 false negatives, that is, cases in which cancer was present but the cytology was reported as negative. In 9 procedures done for benign lesions, the washings were reported as negative. In 3 instances, all cancers of the female pelvis,

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Major Findings: (cont'd)

washings were reported as suspicious when they should have been positive. In summary, it seems that the method devised for identification of tumor cells in washings is approximately 91% accurate.

Complete analysis of the wound washings done on definitive surgical procedures is not available at this time. It is apparent, however, that the association of recurrent disease is not always associated with positive wound washings. In fact, from superficial examination of our data, it would suggest that a positive washing followed by local recurrence of the cancer is about as frequent as a local recurrence in a case with negative washings. Whether or not the cancer cells remaining in the wound are viable and can grow, or whether all of the cells left in the wound can be demonstrated by washing, is not important. It seems to me that the importance lies in the fact that these cells are there. From the reported results in large series of major surgical procedures for cancer, the cause of failure of cancer therapy is the high rate of local recurrence of cancer which is later followed by wide dissemination and death of the patient. In other studies, the transplantability of cancer in humans has been demonstrated, so it would seem reasonable to suspect that if the wound is contaminated by cancer cells, these cells can grow if local and systemic factors are favorable for their growth.

In a large majority of cancer patients, the in-continuity dissection is associated with ulcerative exfoliating primary tumor. This would suggest that possibly the tumor cells that are found in the wound are coming from the exfoliated exposed surface. However, in three melanomas with no ulceration or exposed exfoliating surface, and in two breast cancers with no ulcerative tumor, positive wound washings were obtained. In several radical neck dissections done without resection of the primary, as well as in a number of other instances, positive wound washings were obtained in the absence of the exfoliating primary tumor.

Significance to Cancer Research:

Demonstration of cancer cells in this high proportion of operative wounds again points up the deficiency of our present methods of cancer therapy. The finding of cancer cells in the wound will certainly raise the question as to whether or not local wound therapy would be advisable, and if tried, whether or not it will improve the overall salvage. If, by means of local wound therapy or possibly local wound therapy in conjunction with systemic therapy, local wound recurrences could be prevented, an effective therapy for cancer would be available.

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SERIAL NUMBER

Proposed Course of Project:

During the next few months all the information from these cases will be placed on IBM punch cards, making possible minute analysis of this data. The problem of the correlation between the wound washings and the prognosis should become more clear. At the present time, laboratory studies underway (Project No. C-754) suggest the use of formaldehyde solution as a treatment of postoperative wounds. The safety of this method is being tested and suggest the possibility of using chemotherapy in operative wounds in addition to the surgical procedure in the operating room.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-8-753-C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$50,540	$245,310	$295,850	6	0	6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	6	0	6	2850

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

C-8-753-C

SERIAL NUMBER

Part C: Honors, Awards & Publications

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Paper entitled: Cancer-Cell Seeding of Operative Wounds as a Cause of Failure in the Surgical Treatment of Cancer.
By: Robert R. Smith and Albert W. Hilberg.

To be published in Journal of the Maine Medical Association.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-8-754
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Evaluation of the Nature, Cause, and Means of Preventing Seeding of Operative Wounds using Transplantable Animal Tumors.
PROJECT TITLE

7. Dr. Robert R. Smith
PRINCIPAL INVESTIGATOR

8. Mr. Richard Eck, Dr. Arthur Ship and Dr. William Kramer
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

To produce a laboratory model which would simulate the seeded operative wound and possible systemic dissemination seen in the clinical course of cancer patients. It is the aim of this project to reproduce the factors which are associated with surgical failures and to develop methods of preventing such failures.

Methods Employed:

Most of the previous experimental and clinical work on chemotherapy of cancer has been based upon the effect of drugs on a transplanted tumor

C-8-754

SERIAL NUMBER

Methods Employed: (cont'd)

well established and growing in the new host. The experience of the surgeon in dealing with the surgical treatment of cancer has given rise to a different type of approach since tumor emboli broken off from the primary tumor or its metastases is not an established growing tumor and would therefore be affected by changes in the body's physiology or chemotherapeutic agents in a manner different from that encountered in the internist's studies. It is also possible that the incomplete destruction of tumor by a drug at a time when only a small amount of tumor tissue is present, may produce more effective results by such changes when the body's natural resistance to tumor implantation is still effective. This is in contrast to the effect of a compound in the presence of extensive amounts of tumor tissue. It was therefore necessary to develop methods of artificially seeding an operative wound and of determining the effects of various drugs and procedures upon the ability of these implants to grow and establish themselves. In previous reports, the methods have been fairly well established. V_2 carcinoma grown in rabbits has been placed through a cytosieve and used as an inoculum. Cloudman's S-91 melanoma, K_2 ascites tumor, and Sarcoma 37 have all been used in these studies. The tumor inoculum has been implanted in the animals in the following manner: (1) By producing a surgical wound, such as a flap of abdominal skin; (2) an axillary wound with exposure of the brachial plexus and artery; (3) producing a closed wound on the skin of the mouse by means of an air-bubble technique; (4) by intraperitoneal inoculation; and (5) by intravenous inoculation.

The air-bubble technique as developed by Mr. Eck has made available a new tool for inoculating a large number of animals quickly. This technique simulates the operative wound with cancer fragments by raising a pocket in the subcutaneous tissues on the back of a mouse by the injection of air through the intact skin. After the air has been injected, the tumor inoculum can be injected by needle directly into the pocket, massaged to diffuse throughout the pocket, then by withdrawing the air, the mouse's tissue comes in direct contact with the tumor fragments and thus allows good growth of the tumor inoculum.

These studies were divided into three parts:

(1) The in vitro study consisted of pre-treating the inoculum after it was removed from the donor animal with the drug to be used. The injection of the inoculum at various intervals after treatment, applied in any of the above five techniques, would give information concerning the in vitro toxic effect with direct contact of the compound to the tumor cells.

C-8-754

SERIAL NUMBER

Methods Employed: (cont'd)

(2) In vivo studies, in which the conditions seen in the operating room were more nearly approximated. In these instances the tumor inoculum was placed in the operative wound in the air bubble intraperitoneally or intravenously. In the cases of the operative wound, the inoculum was allowed to remain in contact with the structures for varying periods of time, followed by application of the chemotherapeutic agent. Before each series of studies, the toxicity of the compound being used was studied by use of the compound without tumor inoculum.

(3) Toxicity studies - to test the effect of the drug on normal tissues found in an operative wound.

Findings to Date:

It became apparent that the methods employed required more specific quantitative standardization in order to make possible more precise measurement of effects. Mr. Eck has developed a method for estimating the potency of the ascites inoculum, based upon the time delay before the appearance of ascites in the inoculated mice. Two-fold dilution steps can be readily detected with less than ten mice per set. The method of preparing S-91 melanoma inocula for intravenous injections and counting the number of resulting lung tumors has been standardized. It has been noted by Mr. Eck that a treatment which makes the mice severely sick if the treatment does not have an anti-tumor effect in itself, will double or even triple the number of metastases developing in the lungs. A surprising result was noted in using zymosan as a stressing agent. The exact results with the use of this agent have not been worked out. However, in various experiments zymosan has reduced the number of lung tumors to about 5% in a control group. It has proved effective against both lung and abdominal melanoma even when administered by different routes, but is more effective when given by the same route as the tumor. Preliminary experiments suggest that zymosan is effective only when given within a few days of the tumor implantation.

Arsenic trioxide, as used by many "cancer specialists" for centuries, was studied as a possible means of preventing wound seeding. Dr. Ross Miller did preliminary work on this problem. The compound was found to be so toxic when exposed to such a large surface that its use as a wound treatment seemed of little value. However, the use of arsenic trioxide impregnated threads (in collodian to slow absorption) according to the unpublished data of Dr. S. D. H. Wise, demonstrates that these threads can destroy established accessible tumors. Such threads are effective for a distance of about 3 mm. against S-91 melanoma and S-37 grown subcutaneously in mice. Several mice were cured by a single such application, and others could have been cured

if repeated treatments on the small parts of tumor were continued. Dr. Wise's claim that such arsenic applications are tumor-specific, sparing adjacent normal tissue, was also partly supported by an experiment in which S-37 was established on the soles of mouse feet. In some cases we were able to destroy these tumors with only minimal injury or loss of function of the foot.

The clinical observation of tumor implants associated with suture material prompted exploratory studies of means of preventing such seeding. The use of several protoplasmic poisons made adherent to suture material was tested by Mr. Eck. Ordinary silk sutures saturated with a solution of picric acid was very effective in preventing the contamination of the suture material by tumor implants when the suture was placed through the tumor nodule and then into normal tissue.

Toxicity -

In studying methods of preventing wound implants it becomes apparent early that one of the limiting factors is the toxic effect of the compound used on the animal. It soon became apparent that because of the geography of the wounds, the amount of compound used in the animal with its wound, which is proportionately large in relation to its total body area, is not directly applicable to the human. It was also apparent that locally applied compounds might interfere with wound healing to an extent that their use would be contraindicated. It is possible that the degree of necrosis and effect on nerves or arteries might preclude a drug's usefulness as wound therapy. In order to test the compounds used, the animal was anesthetized, fixed to the operating board, and an axillary wound produced, exposing the axillary vessels and nerves. A wire retractor was made so that the wound was held open, allowing continuous contact of the test material to the axillary structures. Using alpha-peltatin and other podophyllin compounds, complete paralysis was produced when used in very small concentrations. Dakin's solution was the only common drug used in surgery care that caused injury to the vessels which lead to hemorrhage from the intact vessel. Formaldehyde toxicity has been worked out as described in Dr. Ship's report. In preliminary studies, formaldehyde was found to be the most useful and least toxic drug in preventing wound seeding. 0.5% solution sprayed on a wound contaminated with V_2 carcinoma prevented growth of the inoculum in about 100% trials. This was demonstrated in axillary dissections, in abdominal wounds, and by the air bubble technique. More recently Dr. Ship has worked out both the toxicology as well as the effectiveness of formaldehyde in preventing seeding. In preliminary studies he noted that pre-treatment of a wound with 0.1% formaldehyde one-half hour before injection of the inoculum enhanced tumor growth. K_2 ascites tumor grew in the air bubble technique when as few as 4500 cells were injected. In control animals without pre-treatment

with formaldehyde the same number of cells failed to grow; in fact, only two out of four that received 18,000 cells in subcutaneous tissues developed tumors. Using the air bubble technique, it was determined that 1 mgm/gram body weight of mice of formaldehyde was uniformly lethal with a striking uniformity of toxic signs. In thirty seconds the animals became unsteady and at three minutes appeared to be blind. In four-and-a-half minutes they lost their "righting" ability, and in six minutes they were all dead. 5 ml. of 0.1% (or 0.2 mg. per gram of body weight) was not lethal but did make the animals very sick. (This is about ten times the amount used in exploratory clinical studies). Using a slightly smaller dose, or 0.4 mg. per gram of body weight (10 cc. of 0.1% solution) death occurred in twenty-two minutes. When the fluid was injected in the air bubble,the animal being sacrificed within three minutes of the injection, none of the formaldehyde could be recovered from the fluid. The tissues of the air bubble were markedly edematous and apparently all the fluid had been thoroughly absorbed in these tissues. It seemed apparent that the formaldehyde was fixed by the tissues very quickly. By use of intraperitoneal inoculation, it was possible to test the peritoneal fluid for formaldehyde following inoculation. It was noted that the intraperitoneal inoculation of the formaldehyde was tolerated fairly well; however, toxic signs were the same as when injected in the subcutaneous tissue. Spectrophotometer determinations of the peritoneal fluid following formaldehyde injection were performed. It was found that the concentration of formaldehyde did not vary in any of the animals regardless of how long it had been in the peritoneal cavity, and regardless of whether or not the animal had died.

Dr. Ship further tested formaldehyde in wounds by the air bubble technique. He injected known concentrations of S-37 and K_2 tumor cells (500,000, 50,000 and 5,000) by the standard as well as by modified techniques, i.e., in which the tumor cells were suspended in 5 cc. of saline or two concentrations of formaldehyde. 0.125% formaldehyde (5 cc.) was previously demonstrated to be a sub-lethal dose which produced reversible illness in the mice. A smaller concentration (0.05%) was also arbitrarily selected. A large series of animals were treated both with S-37 and K_2 ascites tumor, and in every instance, exposure of the tumor in vitro to these concentrations of formaldehyde for from two to twenty minutes presumably resulted in destruction of the tumor, for in no instance was a successful tumor "take" demonstrated after forty days even though all the controls grew tumor. For purposes of scientific accuracy, the range of formaldehyde concentrations has been extended to include solutions previously demonstrated to partially damage or prevent "take" (0.025% and 0.01%).

As a result of these encouraging studies an attempt has also been made to study the in vivo effect of formaldehyde (all four concentrations) against the K_2 ascites tumor. Here the tumor was

given forty minutes to one hour prior to administering the formaldehyde in the same compartment. A small series of animals had pre-treatment (three hours) with formaldehyde to observe the possible effects of irritation of the host tissues or possible lingering of the solution in an effective concentration sufficient to prevent tumor growth. Finally, several animals were pre-treated with formaldehyde by the intraperitoneal route to investigate any systemic toxic action.

The most recent aspect of study of formaldehyde action has been to separate the tumor cells from their ascitic fluid and to re-suspend the former in saline or excess of ascitic fluid. Here we are simultaneously testing the effects of picric acid and formaldehyde because in preliminary studies picric acid gave more consistent results. We hope to shed light on the mechanism of action of formaldehyde, i.e., possible binding by protein-rich fluid, direct killing of cells, etc.

PART II. (Dr. William Kramer)

The following are the results of the work to date on the effects of chemotherapeutic agents and in particular, tri-ethylene-thiophosphoramide (Thio-TEPA) as wound washing materials and as systemic agents against experimental metastases.

A. Local Effects: (wound washing)

1. Local toxicity - exposure of compound to axillary nerve for five minutes and observation for subsequent paralysis.
   a. Colchicine - toxic to axillary nerve at .10%. Minimal injury at .05%.
   b. Trimethylcolchicinic acid - methyl ether d-tartrate, compound 1136 of Shear (a colchicine analog and reputedly less toxic than colchicine). Toxic to nerve at 1%, non-toxic at 0.5%.
   c. Colchemide (desacetylmethylcolchicine). A newer, less toxic analog, - non-toxic at the only concentration supplied, 0.1%.
   d. Thio-TEPA - 2.5% toxic, 1% non-toxic.

Conclusions: Because of nerve toxicity, colchicine can be used only at very low concentrations. The colchicine analogs are less toxic. Thio-TEPA is the least locally toxic agent of the agents tested.

B. In Vitro tests of tumorcidal effect:

1. Method - Mix Thio-TEPA with tumor inoculum in vitro, then inject into peritoneal cavity of mice and observe for development of ascites (K_2 or S-37) or number of implants (S-91 melanoma). Failure to develop ascites is taken as a

positive result and delay in the appearance of ascites relative to the control is taken as evidence of a partial effect.

2. Results - Colchicine in concentrations which were locally non-toxic was found to be systemically toxic (three of eight animals died) and had no demonstrable tumorcidal effect.

Compound 1136 at its highest non-toxic concentration (0.5%) was not effective over low times of exposure (2,5,10, and 15 minutes), but was effective when left in contact with tumor cells for twenty minutes. It was felt that because of the long period of time necessary for exposure of the drug to tumor, it was not a wise choice for use in the actual wound washing situation in the operating room.

Colchemide was completely ineffective in the concentration used. It is conceivable that at higher concentrations it may be more effective but the drug was not available for further experimentation.

Thio-TEPA was found to be completely effective in 100% of the cases in preventing the development of ascites from K_2 tumor when mixed with the tumor inoculum for two or twenty minutes. 0.5% Thio-TEPA did not prevent the development of tumors when exposure time was only two minutes, but the development of ascites was delayed ten days over the controls. 0.5% Thio-TEPA for five minutes was completely effective in 50% of the cases in preventing the development of ascites and delayed the development of ascites when it did not prevent it. 0.2% Thio-TEPA was 0% effective at two minutes, 25% effective at five minutes, 50% effective at ten minutes and 100% effective at fifteen and twenty minutes.

The results of these studies with Thio-TEPA would seem to indicate that there is a dose-time relationship. Low concentrations will be effective if left in contact with the cells for longer periods of time and vice-versa. 1% Thio-TEPA seems well suited for use as the wound washing material against K_2 tumor inoculum.

Thio-TEPA was also tested against S-91 melanoma <u>in vitro</u> at 0.5% concentration. It was markedly effective against this tumor at all time ranges, including two minutes (controls - 50 spots average; treated for 2 minutes, 1/2 spot average).

C. In vivo testing of Thio-TEPA as a wound washing material:

In this type of an experiment, a wound is made in the abdominal wall and a tumor inoculum instilled. After five minutes the wound is flooded with Thio-TEPA and after another five-minute wait the wound is blotted and closed. The number of animals that develop tumor implants is then noted and the size of the implants is also recorded. In this type of experiment Thio-TEPA is found to be quite effective in preventing implantation of both S-37 and K_2 tumor. The effect is not 100% but varies with the extent of the inoculum which, it should be pointed out, is huge. For example, an inoculum of approximately 50,000 cells will cause tumor implants in approximately 8 out of 9 control animals and 0 out of 10 animals treated with Thio-TEPA. One experiment using the technique of implanting tumor in a subcutaneous air pocket and then instilling Thio-TEPA also demonstrated effectiveness of Thio-TEPA against K_2 tumors.

Projected Studies:

We believe the experiments have demonstrated that Thio-TEPA is effective locally against artificially produced seeded wounds. No further experiments are contemplated at this time. If the projected studies on systemic chemotherapy continue to be encouraging, it would probably not be advantageous to use Thio-TEPA also as a wound wash since some of it would be absorbed and hence decrease the amount that could be safely used systemically. One experiment that will be performed is the determination of the effectiveness of systemic thio-TEPA on a locally seeded wound.

D. Systemic effect:

Thio-TEPA has been used with only sporadic effect against well-established tumors. The in vitro studies described above suggest that it may be of more value used against single cells which have not become firmly established. This would be the situation following surgery with the presumptive presence of tumor emboli secondary to the manipulation. There is no method now available to measure the concentration of Thio-TEPA in the blood. It is obvious, however, that even if it were completely and instantaneously absorbed, the concentration in the arterial blood would be far lower than that found to be effective in the in vitro experiments. This is because Thio-TEPA is a toxic drug and dosage must be limited. Because of the above-described dose-time relationship it is conceivable that Thio-TEPA in low concentrations may still be effective in the blood stream because of the fact that they are left in contact with tumor cells for longer periods of time. The work of Cole in demonstrating the systemic effectiveness of nitrogen mustard against portal vein tumor emboli adds emphasis to this work. Work on this aspect of

tumor chemotherapy has only just begun but the following results have become apparent.

Thio-TEPA, when given intraperitoneally or intravenously, is markedly effective in decreasing the number of "takes" of cytosieve melanoma S-91 injected intravenously one-half hour previously. For example, there are an average of 227 melanoma spots in the control lungs and 13 spots in those treated with intravenous Thio-TEPA, one-half hour after tumor inoculation. Giving Thio-TEPA one-half hour before melanoma inoculation is also effective (average 92 spots per animal) but not nearly as much so as giving the Thio-TEPA after the inoculation. Giving larger doses of Thio-TEPA before inoculation seems to improve the tumorcidal effects. It has been suggested that the effect of Thio-TEPA is merely one of making the animal sick and consequently altering the factors of resistance to implantation in this manner. If this were so, it is doubtful that there would be any difference between injecting Thio-TEPA one-half hour before or one-half hour after the melanoma. The effect seems to be more simply one of adequate level of Thio-TEPA in the circulating serum. Thio-TEPA is equally effective in decreasing the size and number of abdominal melanoma nodules following intraperitoneal inoculation. It is quite effective intravenously, but even more so intraperitoneally suggesting that local as well systemic factors are combined with intraperitoneal treatment of intraperitoneal tumors.

Experiments are now in progress or will be performed in the future to test the following:

A. Confirmation of results using other tumors.
B. Effect of Thio-TEPA at graded intervals of time following tumor inoculation.
C. Effect of multiple doses of Thio-TEPA.
D. Effect of giving Thio-TEPA at varying intervals of time before tumor inoculation.
E. Effect of Thio-TEPA in preventing the metastasis from spontaneously metastasizing tumors.

Significance to Cancer Research:

This project is furnishing tools for the clinical care of cancer patients. Once the significance of the finding of cancer cells in wound washings is established, means to prevent such seeding will be available. If such methods were effective, present-day cancer therapy would be much more effective.

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SERIAL NUMBER

Proposed Course of Project:

It is proposed to continue these studies in order to delineate the factors which might prevent wound seeding.

It seems likely, however, that rather than limit our efforts to local destruction of tumor tissue, a search for a preventive of systemic as well as local seeding will be more important.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-8-754
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,740	$3,110	$10,850	1	1	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-755-C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. The Development of New Methods for Treatment of Lung Cancer Using Regional Chemotherapy: Clinical Research.
PROJECT TITLE

7. Dr. John H. Waite
PRINCIPAL INVESTIGATOR

8. Dr. William Kramer
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

The chief purpose of this project is to improve treatment of those cancers of the lung which are found to be beyond hope of surgical cure by resection.

Methods Employed:

The plan of the study was to combine surgery and chemotherapy. Patients were selected on the basis of their probable resectability. Cases which were found to be inoperable at the time of operation had catheters implanted in the vascular channel supplying the diseased

(Use reverse and additional pages, if necessary)

PROJECT DESCRIPTION (Continued)

Methods Employed: (Cont'd.)

lung. The catheter was then exteriorized to the chest wall. Following the recovery of the patient from the operation, chemotherapy was given to the patient through the catheter. In the study that Dr. Waite did to date, this included merely the use of nitrogen mustard.

Patient Material:

A total of 16 patients have been treated on this project to date; only one during this calendar year.

Major Findings:

As summarized in previous reports, very few patients were available for the study as outlined in that most of them were amenable to possible curative excisional surgery. In the one patient who did receive nitrogen mustard during this calendar year, and is still alive, the palliative effect of the nitrogen mustard given in this way did seem to be quite definite. The study had not progressed to the point, however, where an opinion as to the advisability or the effectiveness of such a regime was established.

Significance to Cancer Research:

It is the opinion of the Surgery Branch that the primary and the important approach to the treatment of lung cancer is probably going to come from a better understanding of the biologic nature and environmental relationship of the disease. The ever-increasing incidence of lung cancer in the adult population makes it imperative that new methods of definitive as well as palliative therapy be developed.

Proposed Course of Project:

Due to the lack of personnel interested and capable of carrying out a thoracic surgical problem, no further work is contemplated on this project at the present time.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-755-C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	0	0	0	0	0	0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	0	0	0

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-756
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Development of New Methods in the Treatment of Lung Cancer Using Regional Chemotherapy: Laboratory Research.
PROJECT TITLE

7. Dr. John H. Waite.
PRINCIPAL INVESTIGATOR

8. Dr. William M. Kramer and Dr. William G. Banfield.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

Using the dog as an experimental animal, the project's aim was to develop and study methods of regional chemotherapy to lung cancers.

Methods Employed:

This is a project of Dr. John H. Waite who has since been transferred to the Public Health Service Hospital at Stapleton, N. Y. The method employed in his studies was the introduction of fine polyethylene catheters at the time of thoracotomy into the bronchial artery or into a branch of the pulmonary artery or pulmonary vein.

(Use reverse and additional pages, if necessary)

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Methods Employed: (cont'd)

Following the surgery, studies were done on the x-ray demonstration of the broncho-pulmonary tree.

Major Findings:

The technique of catheter introduction into the pulmonary tree was perfected during the past year. Very little new information was added to that as reported in this project the previous year. Preliminary attempts at the passing of catheters through the femoral artery with a balloon attached to the tip of the catheter, allowing the balloon to rest just above the diaphragm, produced a block and allowed considerable increase in the amount of diodrast solution seen entering the intercostal vessels. The relation of the intercostal vessels to the bronchial vessels has been adequately demonstrated in the previous studies. It was found at the time of the study that it was necessary to place the balloon just above the celiac axis to allow enough reflux of the dye into the intercostal vessels. Catheters placed at a higher level did not allow the dye to enter the area of the chest occupied by the tumor.

Significance to Cancer Research:

Continuing increase in the incidence and death rate due to lung cancer demands maximal efforts to control the disease. The primary or basic science approach involves efforts to determine the biologic nature and environmental relationships of lung cancer. While of great hope, this research has yet to produce applicable results. A second phase is seen in programs aimed at the discovery and treatment of lung cancer in its earliest stages. This attack has succeeded in curing up to 5% of all patients seen by extirpative surgery, the only curative treatment now known. The third phase of lung cancer research involves improvement of treatment of the disease when it is past its earliest stages, and the large number of well-advanced lung cancer patients now seen provides an urgent motivation for the laboratory investigator to search for an approach such as described in this project.

Proposed Course of Project:

Due to the transfer of Dr. Waite to another Public Health Service hospital, no further work is contemplated on this project at this time.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-756
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$1,630	$640	$2,270	1/3	0	1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	0	1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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(Attachment I)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-8-757C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Prevention of Operative Site Recurrence by Improvement of Biopsy Wound Closure Technique.
PROJECT TITLE

7. Dr. John H. Waite
PRINCIPAL INVESTIGATOR

8. Dr. Robert R. Smith, Dr. Albert Hilberg and Dr. Horace Herbsman
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

The purpose of this project has been to gather some experimental data concerning the validity of biopsy wound closure techniques, to determine if any currently used techniques are satisfactory, and to develop, if necessary, improved techniques for management of biopsy wound closure.

Methods Employed:

Dr. Waite tried on a number of occasions to document the presence of the escape of tumor cells from a biopsy breast wound following closure of the wound after a surgical biopsy of a breast cancer.

(Use reverse and additional pages, if necessary)

C-8-757C
SERIAL NUMBER

PROJECT DESCRIPTION (Continued)

Major Findings:

Three patients were of special interest in this study. These were all advanced breast cancers. Washings were taken from the intact skin prior to and after removal of the primary tumor from the open biopsy wound and then after closure of the biopsy wound. In the three instances, tumor cells were found in the biopsy wound, and in two of the cases, tumor cells were found in washings taken from the skin following closure of the biopsy wound. The lesson learned from this is that the usually adapted continuous interlocking suture used to close a biopsy wound while the radical mastectomy is being done is not sufficient to prevent the escape of tissue juices containing tumor cells.

Significance to Cancer Research:

Wound recurrence following biopsy and local surgery is not uncommon. In treating one of the most common cancers, that of the breast, leading clinics admit to a 12% local recurrence rate. The two most likely factors are inadequate surgical margins and spillage of cells at the time of biopsy. Exfoliated cells have been recovered from skin surfaces by the principal investigator even after more than usually careful attempts at biopsy site closure and sealing off, using conventional techniques. A simple method which would guarantee 100% riddance of skin surface tumor cells would be of immediate value to all surgeons in treatment of many common malignancies.

Proposed Course of Project:

No further work will be carried out under this project. The concept of wound seeding will be covered in other projects.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-8-757C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$1,620	$640	$2,260	1/3	0	1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	0	1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-8-758
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4.
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Prevention of Operative Site Tumor Recurrence by Improvement of Biopsy Wound Closure Techniques: Laboratory Research
PROJECT TITLE

7. Dr. John H. Waite
PRINCIPAL INVESTIGATOR

8. Dr. Horace Herbsman and Mr. Richard V. Eck
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

The purpose of this project is to evaluate methods which have been proposed for biopsy wound closure by laboratory techniques on the experimental animal. A closure method is sought which would prevent the escape of tumor cells from the skin surface into the radical surgery wounds after preliminary biopsy. If currently used techniques are found to be unsatisfactory, new techniques will be developed.

(Use reverse and additional pages, if necessary)

C-8-758
SERIAL NUMBER

PROJECT DESCRIPTION (continued)

Methods Employed:

Using rabbits as the experimental animal, wounds were made in the abdominal wall of the animals which were then sutured in various manners. A dye was then injected under the raised skin flap to test the pressure necessary to cause a leak at the wound site.

Major Findings:

Summarizing Dr. Waite's work, it was determined that it was impossible to develop a fluid-tight closure of a wound without an overlapping clamping of the entire free surface of the wound. All wounds leaked the dye with only moderate pressure.

Significance to Cancer Research:

The problem of the seeded operative wound remains an important reason for failure in the surgical treatment of cancer. If the soiling of wounds could be prevented, the overall survival rate for surgical treatment of cancer would be increased.

Proposed Course of Project:

No further work is anticipated on this project. It is believed that this project represented only a small part of the problem of the seeded operative wound. It is believed that the problem remains one of not trying to prevent the escape of tumor cells in the wound, but of developing methods to destroy any tumor cells that would be left in the main operative wound following the definitive treatment.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-8-758
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$1,630	$640	$2,270	1/3	0	1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	0	1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-759
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Effect of Operation, Anesthesia, and Cortisone on the Incidence of Lung Metastases in Experimental Animals.
PROJECT TITLE

7. Dr. William M. Kramer
PRINCIPAL INVESTIGATOR

8. Dr. William E. Schatten
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

To determine the effect which operation, anesthesia, and cortisone have on experimental metastases. (Stress).

Methods Employed:

Cytosieved S-91 melanoma is injected into the tail vein of brown mice in a dilution calculated to produce pulmonary metastases (easily countable macroscopically) in about 25% of animals. A dose-response curve has already been determined so that the proper number of cells can be injected to produce this effect. One group of animals inoculated in this manner would then be

(Use reverse and additional pages, if necessary)

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Methods Employed: (cont'd)

treated with cortisone 0.5 milligrams per day for three days, including the day of inoculation. This is considered to be the time of maximal adrenal stimulation following an operation. Another group of animals would be anesthetized. A third group would be anesthetized and a xiphoidectomy performed. A fourth group would receive saline. This experiment will then be repeated using a spontaneously metastasizing tumor.

Major Findings:

The project has not yet begun.

Significance to Cancer Research:

The occasional problem of sudden appearance of diffuse metastases shortly after definitive or palliative cancer surgery is perplexing. Despite lack of proof, the surgeon cannot help but feel that his intervention may have precipitated the untoward events. Manipulation of the tumor has been shown by Tyzzer, Knox, and Marsh to cause an increase in the number of experimental metastases. The continual seeding of malignant cells into the portal circulation has been demonstrated. Apparently not all the cells "take", since not all patients with seeding into the portal system developed liver metastases. It is conceivable that the operation per se has altered the resistance of the patient so that free tumor cells are more likely to implant successfully. The outpouring of adrenal hormones known to occur for the first few days postoperatively might be one mechanism by which this could occur. Baserga and Shubik have shown that there are latent lung metastases present after amputation of a tumorous mouse tail. Giving cortisone following operation causes a much greater number of these to take than in a control group. These workers have also shown cortisone to be effective in inducing or increasing metastases from mammary adenocarcinoma and methyl cholanthrene-induced skin and subcutaneous tumors. Pomeroy has demonstrated that cortisone will increase a number of metastases following intracardiac injection of K_2 tumor. Agosin has demonstrated the establishment of metastasizability of an adenocarcinoma in 40% of animals treated with cortisone where previously no spontaneous metastases occurred. To these findings may be added the fact that treatment with cortisone permits transplantation of homologous and even human heterologous tumor where formerly these tumors would not survive. Despite this, cortisone, especially in large doses, has frequently been noted to have an inhibitory effect on the primary tumor. In fact, the local tumor has been reported to regress in the same animals which have been stimulated to increase metastases by cortisone. A review of the

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SERIAL NUMBER

literature has not revealed any studies on the effect of operation, or operation and anesthesia on the incidence of metastases. Possibly the operation or even the anesthesia with resultant stress reaction and outpouring of adrenal cortical hormones might facilitate the implantation of latent metastases, or perhaps the operation or anesthesia may be incriminated through some other mechanism. It is the purpose of this experiment to test this hypothesis.

Proposed Course of Project:

This is covered fully in the section on "Methods Employed".

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-759
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$2,430	$960	$3,390	1/2	0	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	0	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-760
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Intestinal Mucous Membrane Grafts to the Mouth.
PROJECT TITLE

7. Dr. Horace Herbsman.
PRINCIPAL INVESTIGATOR

8. Dr. Alan S. Rabson and Dr. William E. Schatten.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

The replacement of oral mucous membrane has long been a surgical problem. The correction of traumatic oral deformities, congenital and developmental malformations of the mouth, and defects created by surgery have all made necessary the development of a reparative process which would be both functionally and cosmetically adequate. With the recent advances in radical intra-oral surgery for cancer, the need for this has become even more acute; the resection of the buccal mucosa or maxillary antrum leaves large intra-oral areas devoid of epithelial surface and to preserve appearance and function, a suitable restoration must be accomplished.

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

Objectives: (cont'd)

Esser, in 1917, successfully used a free split-thickness skin graft to replace the deficient oral mucous membrane. This method was later modified by other surgeons but the use of skin grafts has remained the most effective method thus far to replace oral mucous membrane. However, this method is not without disadvantages. The skin is dry, has poor elasticity and mobility after transplantation, and is physiologically not adequate replacement. It is non-secreting and the epithelial cells become macerated, exfoliated, and produce a foul smelling discharge. Moreover, the skin is usually hair-bearing which is disadvantageous for intra-oral use, and also undergoes considerable fibrosis and shrinkage, frequently resulting in disability in speech and deglutition.

Free mucous membrane grafts might afford more satisfactory results, but their use has never been explored in intra-oral reconstruction, probably due to inadequate source of mucous membrane. Small free mucous membrane grafts have been used successfully to repair bucco-gingival defects and to line orbital cavities following ocular enucleation. These have been obtained from buccal mucosa, conjunctiva, vagina, and even rectal mucosa.

Methods Employed:

The present study is concerned with evaluating the feasibility of grafting intestinal mucous membrane to the mouth for the purpose of replacing large oral mucosal defects. A loop of small intestine is resected in the dog, the mucosa stripped from the muscularis, and the free mucous membrane graft thus obtained used to line a previously prepared oral cavity defect in the same dog. A free split-thickness skin graft is also taken from the same animal and used in a similar manner to line another cavity in the same dog's mouth. A control of skin in each dog is then able to be compared to the mucous membrane graft. After a suitable length of time, the animals are sacrificed and the grafts examined histologically.

Major Findings:

The proposed procedure was performed on eight dogs. A stent of dental compound was used to hold the graft in place. Biopsies after several days showed considerable ulceration of the mucous membrane grafts. The skin grafts also showed only partial "takes". Patch grafts of jejunum mucous membrane were then placed in the dog's mouth and biopsied at intervals of several days. Viable jejunal mucous membrane was noted at four weeks, indicating that

C-10-760
SERIAL NUMBER

Major Findings: (cont'd)

intestinal mucous membrane can survive in a dog's mouth. Technical aspects of the procedure were then revised, and intestinal mucous membrane transplanted to the mouth of the five dogs. These dogs will be sacrificed after three months and the grafts examined histologically.

Proposed Course of Project:

To follow grafts already in place and to try to modify the technique so that the mucosa will grow in its new location.

Significance to Cancer Research:

If a mucous secreting surface could be transplanted into intra-oral areas following radical resections of head and neck cancers, it would improve the operation and make functional results better.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-760
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$1,620	$640	$2,260	1/3	0	1 3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	0	1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-761
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4.
SECTION OR SERVICE

5. Memorial Hospital & Sloan-Kettering Institute
LOCATION (IF OTHER THAN BETHESDA)

6. Blood-Pressure Servo-Regulator.
PROJECT TITLE

7. Dr. John H. Waite
PRINCIPAL INVESTIGATOR

8. John Laughlin, Naomi Sager, William Peppall and William Howland.
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

To construct a machine for automatic regulation of blood pressure in shock due to vasodilation, and in hypotensive anesthesia.

Methods Employed:

The mean arterial blood pressure is measured directly with a transducing manometer. Appropriate vasopressor (norepinephrine) or vasodepressor (arphonad) solution is administered continuously intravenously by a variable speed infusion pump. The pump speed

(Use reverse and additional pages, if necessary)

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SERIAL NO. NY

Method Employed: (cont'd)

is regulated by a signal generated by a servo-loop. The servo-loop signal measures the difference between the blood pressure measured and the blood pressure clinically desirable for the patient being treated. The therapist thus needs only to set the control panel for the desired blood pressure, and the auto-regulated machinery administers blood pressure regulating substance as needed.

Major Findings:

The blood-pressure servo-regulator apparatus was constructed and installed in the laboratories at the Memorial Center for Cancer Research. The practicability of the machine in post-operative recovery room use has not been conclusively demonstrated. This part of the project has been taken over by the Memorial group. The N. C. I. Surgery group has not had anything to do with the testing or the clinical use of this piece of apparatus.

Significance to Cancer Research:

The machine, if successful, will be an aid in maintaining blood pressure in patients with neurogenic shock due to radical surgery or drug reactions. It will also provide a method for controlled lowering of blood pressure (hypotensive anesthesia) in patients undergoing radical surgery for cancer.

Proposed Course of Project:

This project is completed as far as the National Cancer Institute's participation is concerned.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-761
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	0	0	0			0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	0	0	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-762-C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Blood Volume, Plasma Volume, Red Cell Mass and Total Circulating Plasma Protein and Electrolytes in Cancer Patients During Preoperative Bowel Preparation and the Period After Radical Surgery.
PROJECT TITLE

7. Dr. Robert A. Milch
PRINCIPAL INVESTIGATOR

8. Dr. Jesse L. Steinfeld and Surgical Staff, N. C. I.
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

Although considerable data have been presented from several laboratories concerning the metabolic responses to surgery and other forms of trauma, little attention has been directed to metabolic changes in patients undergoing various preparatory procedures during the immediate preoperative period, the operative and immediate postoperative period. This is particularly true of female patients undergoing extensive preparation of the bowel for pelvic surgery prior to contemplated exenterative procedures. The objectives of the present study are to determine the above parameters serially in patients undergoing "vigorous" as opposed to "mild" preparation of

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

Objectives: (cont'd)

the gastrointestinal tract for both pelvic and extra-abdominal surgical procedures.

Methods Employed:

Injection of known small amounts (approximately 10 microcuries) of I^{131} labelled human serum albumin and measurement of its dilution at 15 and 30 minutes after injection in both whole blood and plasma. Coupled with serial determinations of the plasma concentration of red cells (hematocrit), plasma protein, and serum electrolytes, it will be possible to calculate the total amounts of these various substances which are circulating in the plasma and the effect of bowel preparation of them from calculated data on red cell mass, plasma volume and blood volume.

Patient Material:

Patients undergoing major surgery by the N. C. I. Surgery Branch will be studies.

Major Findings:

Three patients have been studied in whom daily blood volumes and electrolyte determinations have been made, both prior to and during the course of rigorous preoperative bowel preparation. Plasma volume and circulating electrolyte pools in these patients, all of whom were clinically in "good shape", remained essentially constant despite the patient's complaint of feeling "tired and washed out" and clinically presenting a picture of depletion. The data available would seem to contra-indicate some of the published data which implied that the rigorous catharsis lead to a depletion of electrolytes.

Significance to Cancer Research:

In order to improve our methods of treating cancer, more precise information is necessary concerning the metabolic status of cancer patients coming to surgery. This project should help the surgeon prepare patients for major surgery.

Proposed Course of Project:

It is hoped that during the next six months, this project will be completed. It is anticipated that it may be necessary to limit the scope of this study to the serum electrolyte components rather than a total body electrolyte study.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-762-C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$1,620	$640	$2,260	1/3	0	1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	0	1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-763
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. The Effect of Prednisone (Metacorten) Upon the Healing of Wounds.
PROJECT TITLE

7. Dr. Lester M. Cramer
PRINCIPAL INVESTIGATOR

8. Dr. Eli M. Nadel
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

Because of its greater anti-inflammatory action and its lesser effect upon ion metabolism, prednisone is rapidly replacing compounds E and F in a large segment of clinical medicine, especially in the management of rheumatoid arthritis, chronic pulmonary asthma, and many ocular and dermatologic conditions. The inhibitory effect upon wound healing of compounds E and F is well documented. It becomes imperative to know if this new compound will have greater wound healing inhibitory effect as might be expected from its greater effect upon inflammation.

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

PROJECT DESCRIPTION (Continued)

Methods Employed:

280 C57Bl mice were divided into two groups of 140 each, and then each of these subdivided into 14 groups of 10 each. One-half of all the animals were given prednisone .25 mgm/20 gm. daily for 5 days before wounding, the other one-half served as pair-fed controls.

The wound consisted of a 2 cm. incision in the anterior wall of the distal stomach. 5-0 nylon thread was used to close the wound in linear fashion, using a continuous horizontal mattress suture for serosal coaption. Prednisone was given daily postoperatively, and all animals were weighed daily. A group from each of the prednisone and control groups was sacrificed on each day for fourteen days, and the wounds studied in two ways: (1) Strength of wound as determined by introduction of compressed air until the suture line bursts. (2) Histologic character.

Major Findings:

Study of the inflammation pressure in millimeters of Mercury (mm Hg) necessary for bursting the wound was measured from the day of incision and suture to the fourteenth postoperative day, and the wounds were studied histologically after formalin fixation. In untreated mice the bursting strength curve was similar to that reported for the untreated rat. The curve for the prednisone-treated animals was essentially similar to that of untreated mice with the exception of the first postoperative day, when the wound in prednisone-treated animals appeared less able to withstand internal pressure. In the prednisone-treated animals fewer inflammatory cells, less edema, and relatively fewer fibroblasts were seen on the first postoperative day. By the fifth postoperative day granulation tissue was evident in both treated and untreated groups. However, the amount of granulation tissue appeared to be less, the connective tissue stroma looser, and the fibroblasts plumper in animals receiving prednisone. By the ninth postoperative day, wounds from both groups had healed. However, the amount of granulation tissue and the degree of scarring and deformity appeared markedly less in the prednisone-treated group. Thus, prednisone modified the histologic response of healing in the stomach, but did not significantly alter or weaken the strength of the wound after the first postoperative day as measured by bursting pressure. Mucosal and serosal healing was not delayed after prednisone, and the decrease in the connective tissue response made for a less bulky and less deforming scar.

C-10-763
SERIAL NUMBER

Significance to Cancer Research:

The use of new steroids as a palliative agent in the treatment of cancer produces serious problems in the surgical management of patients, especially if it could be shown that these drugs do inhibit wound healing. The results of this study would seem to imply that the wound healing, as measured by the bursting strength of the artificially induced stomach wound, would not be a factor in the clinical management of these patients.

Proposed Course of Project:

The project is considered completed.

The results of this study have been completed and the manuscript entitled: "The Effect of Prednisone Upon Gastric Wound Healing" will be submitted to the Annals of Surgery for publication in the near future.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-763
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$2,430	$960	$3,390	1/2	0	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	0	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-8-764
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. The Transplantation of "Conditioned" Endocrine Tissue.
PROJECT TITLE

7. Dr. Lester M. Cramer
PRINCIPAL INVESTIGATOR

8. Dr. William Schatten, Dr. William Mohler and Dr. Jesse Steinfeld
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

To demonstrate modifications in the immune responses to homografted tissues by modifying the antigenicity of the donor tissue.

Methods Employed:

Fresh thyroid tissue was obtained from rabbits less than 48 hours old and explants of these tissues were grown in media consisting of Eagle's nutrients and serum of the recipient rabbit. It was planned that after developing the tissue culture thyroid explant and demonstrating

(Use reverse and additional pages, if necessary)

PROJECT DESCRIPTION (Continued)

Methods Employed:(continued)

its functional ability, the original plan was to transplant the tissue culture explant back into the abdominal wall of the rabbit which had been thyroidectomized two weeks previously. It was planned to use I131 uptake as the criterion for determining functional ability of the explant.

Major Findings:

This work has developed into a tissue culture study, part of which is being done by Dr. William Mohler. This will be covered by a project report from his laboratory. The project as envisioned by Dr. Lester Cramer a year ago never did develop because the tissue culture growth of the thyroid tissue was never satisfactorily obtained. No uptake studies were attempted.

Significance to Cancer Research:

In the treatment of cancer of endocrine glands it is often necessary to remove the entire gland. This necessitates that the patient continue taking substitution drug treatment for the remainder of life. If a method of producing a functioning homologous graft could be developed, possibly using tissue cultured grafts, considerable benefit to clinical management would be obtained.

Proposed Course of Project:

No further work is contemplated on this particular project at this time because of transfer of personnel.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-8-764
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$1,620	$640	$2,260	1/3	0	1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	0	1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-765
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Effect of Carcinogenic Agents using a Bronchial Pouch Preparation in the Dog.
PROJECT TITLE

7. Dr. John H. Waite
PRINCIPAL INVESTIGATOR

8. Dr. W. C. Hueper and Dr. William Banfield
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

(1) To introduce and utilize the blind pouch technique into bronchogenic carcinoma carcinogenesis study. Results may be added to current evidence concerning the etiology of human lung cancer.

(2) To provide a satisfactory large animal tumor for treatment studies.

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

PROJECT DESCRIPTION (Continued)

Methods Employed:

As performed at present the pouch is fashioned from the left lower lobar bronchus. A left lower lobectomy is first performed, amputating the bronchus more distal than usual. The lower lobe bronchus is then re-amputated at a higher level, severing the cartilagenous ring and endobronchial structures, but leaving the peribronchial structures intact as a vascular pedicle. The left main bronchus is sutured as are both ends of the pouch, forming in effect a pseudocyst. The end of a fine polyethylene catheter is implanted into the lumen of this pouch, and the other end of the catheter is exteriorized through the chest wall where it is buried beneath the skin, available for injection purposes.

Suspected carcinogenic agents will be injected into the bronchial pouch via the polyethylene catheter at regular intervals. Suitable controls where the vehicle only is injected will be included. Serial roentgenograms will be taken to detect and follow the progress of any left hilar radiodensity which may develop. The animals will be followed for four to five years, after which time they will be sacrificed. It is proposed to study the following agents initially: 3,4 benzpyrene, tobacco tar, and internal combusion engine exhaust concentrate.

If sufficient animals and facilities are available, rates of uptake of radio-labelled samples of these compounds may be studied. Further experiments will be conducted to attempt production of a bronchial pouch or bronchostome continuous with the skin surface for carcinogenesis studies.

Major Findings:

Approximately twenty dogs have been prepared with an isolated, closed viable segment of the left lower lobe bronchus. Approximately one-fourth of these dogs are control dogs to study the fate of the bronchial pouch preparation. Approximately one-fourth of the dogs have a benzpyrene pellet implanted in the pouch, one-fourth have a diesel exhaust soot compound injected into the bronchial segment, and one-fourth have a tobacco tar preparation in the bronchial segment. In general, it is planned to study these animals for five years. The development or failure to develop a carcinoma in these pouches will be of carcinogenic significance, and if carcinoma can be regularly produced with one or more of these compounds, the resulting animal would be of value in cancer therapy studies. Each group will be described with reference to the method of preparation, the maintenance studies required, and plans for the future.

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SERIAL NUMBER

Major Findings: (cont'd)

A group of control animals have been prepared in the manner described with bronchial pouch preparation. Several of these have been sacrificed and at autopsy the bronchial wall was found to be lined with normal bronchial mucous membrane without infection or loss of mucous membrane. In all, 13 animals were made with preparation. Studies of this preparation will form the control for the other groups to follow. These animals will be followed over the next two years by people in Dr. Hueper's section.

The second group of benzpyrene dogs have been prepared. In each of these dogs, a small gelatin capsule containing 3,4 benzpyrene and cholesterol mixture was prepared by Dr. Hueper according to stated specifications. Four animals had this compound placed in their prepared pouch. These animals are still under observation. In Group 3, the tobacco tar series, the tobacco tar preparation consists of a crude tar extract which is to be inoculated into the pouches at three month intervals. Five animals were prepared in this way and mixtures of the tar extract have been injected at repeated intervals through a polyethylene catheter. Observations are being made on these animals. In Group 4, diesel engine exhaust soot group, five animals were included. They received a preparation of 1.5 grams of diesel engine exhaust soot mixed with 4.5 cc of tricaprylin. These dogs received the material through a catheter which was placed in the pouch and allowed to remain in the subcutaneous tissues of the animal.

Significance to Cancer Research:

To date the majority of evidence purporting to bear on the genesis of human bronchogenic carcinoma is adduced from the ability of a suspected carcinogenic agent to produce a tumor when painted on the skin of a rabbit or mouse. In interpreting the results of these experiments, not only must the heterogenicity of the experimental animal with respect to humans be considered, but dissimilarity between epidermis and bronchial epithelium plays an important role. Exposing the experimental animal to an atmosphere laden with the suspected carcinogenic agent has almost always been unsuccessful in producing tumors. This had lead to the conclusion by some investigators that the agents are not respiratory carcinogens and by others that the animals' respiratory defense (the cough, the dilution of the carcinogenic agent in the respiratory secretions, and the action of cilia) are so efficient as to make exposure to the carcinogenic agent negligible using this technique. There is much logic to support the second hypothesis, and a good method to provide contact with a strong concentration of the suspected agent over a prolonged period of time and against which contact the animal may not have an effective defense mechanism might be of some value in settling this point.

Significance to Cancer Research: (cont'd)

We feel that a study on the uptake of radioactive benzpyrene from our pouch preparation alone might yield sufficient data to justify the project.

Proposed Course of Project:

This project, carried on by Dr. Waite until his transfer to another USPHS hospital in October, is being completed by personnel within Dr. W. C. Hueper's group. The animals are being cared for by Dr. Hueper and it is our understanding that the Surgery Branch will not be involved in further studies of this project at this time.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-8-765
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$1,630	$640	$2,270	1/3	0	1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	0	1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-766-C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Combination Therapy of Esophageal Carcinoma.
PROJECT TITLE

7. Dr. John H. Waite.
PRINCIPAL INVESTIGATOR

8. Dr. J. Robert Andrews and Dr. Andrew G. Morrow
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

This project will attempt to develop a satisfactory method for treatment of squamous cell carcinoma of the esophagus. Improvement of operability, resectability, and cure rate, will be sought by a program of preoperative rotational supervoltage radiation. A byproduct of the study will be the availability of the recently irradiated esophagus for intensive pathologic study to evaluate the effects of rotational supervoltage on this tumor. By use of tube feedings during the entire period of preoperative radiation an attempt will be made to improve caloric intake and reduce the incidence of hemorrhage and perforation during the course of therapy.

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

Methods Employed:

(1) Complete workup to determine the extent of tumor. Patients with metastatic carcinoma or with regional invasion of a vital structure, such as heart or lung, will not be suitable.

(2) Insert plastic Levin tube at esophagoscopy to provide maintenance and improvement of nutrition. Consider insertion of a nuchal esophagostomy tube if patient develops hypopharyngeal irritation due to the indwelling Levin tube.

(3) Radiation plan of study by Dr. Andrews as coinvestigator. In general, he plans to give external two million volt therapy by rotation technique, beginning with low doses and increasing gradually to a total of 6,000 to 7,000 "r" axial dose over a period of six to seven weeks. If difficulty in achieving a satisfactory vertical width of port is encountered, the possibility of delivering supplemental gamma rays from an available intra-luminal source will be considered. The presence of the indwelling Levin tube would be ideal for this technique.

(4) Surgery (with Dr. Morrow) about four weeks after completion of radiation. If resectable, total esophagectomy with dissection of the regional nodes will be performed with immediate or delayed reconstruction of gastrointestinal continuity.

Evaluation criteria will include (1) changes in tumor size by serial roentgenography during radiation, (2) extent of radiation reaction and tumor fixation at the time of surgery, (3) extent of tumor and lymph node metastases by pathological examination of surgical specimen, and (4) clinical course of patient after treatment.

Patient Material:

Patients with esophageal carcinoma who would fit into this project were difficult to obtain. No new cases were added to the study during the year 1956.

Major Findings:

The use of combined supervoltage x-ray therapy followed by surgical resection has not been established. From the general experience gained, it seems that the use of radiation for esophageal carcinoma in certain cases is very effective in eradicating the disease. In fact, in several of the patients treated, the carcinoma has been observed to be completely eradicated.

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SERIAL NUMBER

Major Findings: (cont'd)

However, the post-radiation sequlae and the surgery problems related to it, have been great and have not been worked out. The impetus to this study was carried by Dr. John H. Waite. Interest in the combined approach by other branches has not been too apparent. From the surgeon's standpoint, the use of pre-operative radiation in esophageal carcinoma remains an important and unanswered question. It is one that I hope this branch will be able to help answer.

Significance to Cancer Research:

Carcinoma of the esophagus is the fifth highest in frequency among internal cancers in adult males. This frequency and the poor results available from present methods of treatment make it an ideal subject for cancer research. Adequate surgery and adequate radiation each fails in its own way to control more than 2% of the entire group of patients presenting themselves with esophageal carcinoma. The chief advantage of supervoltage rotational therapy x-ray is appearing to be better immediate palliation, although the incidence of eventual recurrence and death probably will not be much lower. Combination of surgery, supervoltage and rotational therapy is being tried at several centers, utilizing surgery as the primary treatment and roentgen therapy as a "mop up" treatment. For the reasons already enumerated, the principal investigator feels that the initial use of radiation may provide superior results.

Proposed Course of Project:

During the foreseeable future in the absence of a surgeon competent and interested in thoracic surgery, this project will by default be postponed.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-766-C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$1,620	$640	$2,260	1/3	0	1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	0	1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-767-C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4.
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Physiologic and Environmental Factors Associated With Hypothermic During Extensive Surgical Procedures.
PROJECT TITLE

7. Dr. Horace Herbsman.
PRINCIPAL INVESTIGATOR

8. Dr. Robert R. Smith and Dr. Clarence L. Hebert.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

It has been shown that hypothermia may be induced in patients with resultant physiologic changes advantageous to the performance of certain major surgical procedures. However, spontaneous hypothermia has recently been noted to occur during extensive surgical procedures in the Clinical Center operating rooms. Associated with this sudden decrease in body temperature has been a concomitant decrease in the clinically detectable blood pressure, falsely suggesting to the operating team that blood replacement has been inadequate. The objective of this study is to further evaluate

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

Objectives: (cont'd)

this problem of spontaneous hypothermia, investigating such contributing factors as the patient's age, weight, body build, state of hydration, disease being treated, previous treatments, premedication, anesthetic agents employed, operating room temperature and humidity, degree of surgical draping, and the surgical procedure being performed. The advantages and disadvantages of this spontaneous hypothermia in relation to the surgical procedure will also be evaluated. If found advantageous, methods of uniformly producing hypothermia will be investigated. If found disadvantageous, methods of preventing the spontaneous occurrence of hypothermia will be studied.

Methods Employed:

Temperatures of the patient from various body surfaces will be recorded simultaneously with a multi-channel thermocouple which will also record the prevailing room temperature. Various physiologic determinations on the patient will be obtained using standard methods.

Patient Material:

During the past year, continued experience with the spontaneous development of hypothermia has been accumulated. The material for the project will be obtained during operations on patients admitted for other projects.

Major Findings:

The development of moderate hypothermia in the air-conditioned operating room as experienced in our long major surgical procedures has been observed a number of times. The development of this hypothermia has been documented by intra-esophageal temperatures and correlated with the measured evidence of shock but different from ordinary hemorrhagic shock in that the pulse remains slow. The peripheral-vasal constriction leads to false blood pressure determination. The patients remain dry and do not appear to be in shock. The problem of over-transfusion in these cases has demonstrated the necessity of more careful measure of actual blood loss and adequate replacement.

Significance to Cancer Research:

The information noted in this study is helpful in making major surgical procedures safe and more successful.

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SERIAL NUMBER

Proposed Course of Project:

The problem with the changes in the physiologic state of the patient during anesthesia given for long operative procedures remains an important study. The value of limited hypothermia, that is, temperature down to 30-32° C, raises the question of whether or not this state would possibly be beneficial to a large majority of patients during extensive major surgery. The question of inducing this state voluntarily rather than depending upon large volumes of cold intravenous solutions and the air-conditioned exposure of large operative wounds to lower body temperature will be studied.

During the coming year, further studies upon this will be undertaken.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-767-C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$1,620	$640	$2,260	1/3	0	1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	0	1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-7680
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. The Effect of Hypophysectomy on Uropepsin Excretion.
PROJECT TITLE

7. Dr. James R. Jude
PRINCIPAL INVESTIGATOR

8. Dr. Delbert M. Bergenstal and Dr. Peter B. Olch.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

To determine the effect of hypophysectomy on the hypothalamic-pituitary-adrenalcortical-gastric pathway as evidenced by the excretion of uropepsin.

Methods Employed:

A pepsin substance with proteolytic activity on highly acid substrate was discovered in the urine in 1860 by Brücke. Grutzner in 1881-82 confirmed its presence by means of a fibrin method for

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

Methods Employed: (cont'd)

gastric pepsin. The name, uropepsin, was suggested by Bendersky. Bucker, Gray, and Mirsky are the principal contemporary investigators of uropepsin excretion. Since 1948 several articles by these investigators have appeared. An accurate relationship of uropepsin to gastric pepsin output has been found by Janowitz. Seymour Gray has studied the adrenal influences on the gastrointestinal tract and peptic ulceration in Addison's disease by evaluation of 24-hour uropepsin excretion. He has established normal values by his method of uropepsin excretion and the magnitude in adrenal insufficiency, Cushing's disease and peptic ulceration.

24-hour uropepsin excretion will be determined pre- and post-operatively on patients subjected to a hypophysectomy for treatment of metastatic disease, chiefly that from breast carcinoma.

Patient Material:

Hypophysectomized patients of the Endocrine service will supply material for this study.

Significance to Cancer Research:

The project will further delineate the biologic changes produced by hypophysectomy, a procedure being used more frequently as a palliative treatment for advanced breast cancer.

Proposed Course of Project:

To collect data during the next year to establish the effect of the procedure on uropepsin excretion.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-768-C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	0	0	0	0	0	0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	0	0	0

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-769C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Circulating Plasma Lipids in Postoperative Patients.
PROJECT TITLE

7. Dr. Robert A. Milch
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives:

To study changes in plasma lipids in patients following radical surgery for cancer.

Methods Employed:

Study of serum lipids, plasma volume, and hepatic function in the postoperative patient will be performed by determining plasma volume, using radioiodinated human serum albumin, total lipids cholesterol, cholesterol esters, lipid phosphorus and total fatty acids. Cholesterol fatty acids, phospholipid fatty acids and "neutral

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

Methods Employed: (cont'd)

fats", including free fatty acids, will be calculated on the basis of these data. Since it has been demonstrated that there is a postoperative interference with BSP excretion, liver function tests specifically, bilirubin thymol turbidity, etc, will also be determined. The plan is to estimate each of the above parameters on the day before surgery, the morning of the operative procedure, immediately after termination of the procedure and on the first three or four postoperative days, in an attempt to discover, if possible, the presence of any plasma alterations which might be associated with the previously documented loss of body fat in the postoperative state.

Major Findings:

To date 14 patients have been studied. Four patients underwent cerebral arteriography without other procedures. The remaining ten patients underwent a variety of operative procedures. Blood samples were obtained from each patient on a daily basis throughout the first two weeks of their postoperative course.

Preliminary analysis of the data shows a profound fall in all measured components of plasma lipids in the postoperative period in all but two of the patients. The fall does not appear related to the state of the patients' hydration, nor to their daily caloric intake. The two patients demonstrating a rise in plasma lipid levels were jaundiced; one patient demonstrated an initial decrease in plasma lipid levels in the immediate postoperative period with a subsequent rise coincident with the development of jaundice.

Significance to Cancer Research:

Delineation of the metabolic changes occurring following major surgery will help delineate the problems of surgical therapy of cancer. In addition, it is hoped that an understanding of the metabolic changes occurring with surgery will make such surgery more safe in the postoperative management on a rational basis.

Proposed Course of Project:

It is planned to further delineate the changes noted above as well as further studies on the plasma lipids of all patients.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-769C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	0	0	0	0	0	0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	0	0	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-770
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4.
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Study of the Effect of Growth Hormone on Cartilage Transplants and of the Survival and Growth of these Transplants.
PROJECT TITLE

7. Dr. William E. Schatten
PRINCIPAL INVESTIGATOR

8. Dr. Delbert M. Bergenstal, Dr. William Kramer and Dr. Richard Swarm
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

(1) To study the biological survival of cartilage grafts.
(2) To study the effect of growth hormone administration on growth of epiphysial transplants.

Methods Employed:

Hypophysectomized rats are used in this study because they are sensitive to small amounts of growth hormone and because non-treated hypophysectomized animals are better controls than normal rats in this experiment. Three groups of rats are being studied, autografts, homografts, and heterografts. The cartilage grafts are transplanted

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

Methods Employed: (cont'd)

into the axilla and abdominal wall. Rats in each group are sacrificed at weekly intervals. On the four days prior to sacrifice, S-35 is injected intraperitoneally. After the animals are sacrificed, the cartilage grafts and the animal's own costal and tibial cartilages are removed for study. The primary method of studying activity of the cartilage is by determining the S-35 uptake of the cartilage grafts as expressed in counts per 100 mg. of dry cartilage per minute. In addition, epiphysial transplants are studied by actual measurements of the grafts in millimeters to compare the amounts of growth. The transplants are compared with animal cartilages that are not transplanted and also with the transplants of a control animal that did not receive growth hormone.

Major Findings:

It has been demonstrated that heterografts of costal cartilage, transplanted from rabbits to rats, are not biologically viable at the end of two weeks following grafting. Autografts are viable and can be demonstrated to be so as late as twelve weeks following grafting. Following stimulation of S-35 uptake by growth hormone administration, autografts exhibit as much activity as that of the host's own cartilage. Homografted cartilages fall in between the above-mentioned groups. At the end of five weeks, homografted cartilages are biologically viable but they are not as active in their response to growth hormone and in their uptake of S-35 as are the host's own cartilages.

At the present time homografted cartilages at ten and fifteen weeks following transplantation are being studied to see if these cartilages will be biologically viable.

At the present time costochondral epiphysial transplants have been autografted and growth hormone administered locally in the regions of some of the transplants. Five weeks following transplantation, the transplants and in situ control costal cartilages that were measured and marked at the time of transplantation will all be measured to observe the amount of actual growth of the epiphysial transplants and the amount of stimulation that was effected by growth hormone administration.

Significance to Cancer Research:

Reconstruction of surgically created defects with cartilage grafts is a common procedure. There is still great controversy as to whether or not cartilage homografts survive homotransplantation. It is generally agreed that cartilage homografts exhibit functional

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SERIAL NUMBER

Significance to Cancer Research: (cont'd)

survival. The difficulties in determining whether or not there is biological survival or primarily because of the low metabolic activity of cartilage. Therefore, at the present time, there have been no conclusive studies that have answered the question of biological survival.

It is agreed that the uptake of sulphur by cartilage is dependent upon a vital process and only viable cartilage will take up sulphur and use it in the formation of chondroiten-sulphuric acid. It is believed that using S-35 uptake as a criterion and the ability of this uptake to be stimulated by growth hormone administration will offer conclusive evidence as to whether or not there is biological survival of cartilage homografts.

A study of growth of epiphysial transplants is of obvious importance in the reconstruction of areas such as a mandible that has either been surgically removed or affected by disease process in a patient during his years of active growth. In the past, reconstruction in such an area in an actively growing patient has resulted in disappointment after the patient reached maturity because of lack of change in size of a graft. Transplantation of an epiphysis might result in continued growth of the transplant. It now appears that growth hormone will be available in the future that will be effective in humans. A study of the effect on the growth of epiphysial transplants by growth hormone, therefore, seems indicated.

Proposed Course of Project:

Studies on autografts, homografts, and heterografts of cartilage transplants have been completed with the exception of homografts transplanted for ten to fifteen weeks. Studies on the growth of epiphysial transplants and the effect of growth hormone have been performed and the animals will be sacrificed shortly and the grafts measured.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-770
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$2,430	$960	$3,390	1/2	0	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	0	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16 and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-771
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Experimental Reconstruction of the Extra-hepatic Biliary System.
PROJECT TITLE

7. Dr. William E. Schatten
PRINCIPAL INVESTIGATOR

8. Dr. H. Herbsman, Dr. R. Swarm, Dr. L. Cramer and Dr. R. Smith
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

To develop a successful method of repair of common bile duct defects.

Methods Employed:

Adult mongrel dogs are selected as experimental animals. A cholecystectomy and ligation of the common bile duct is performed in order to closely simulate conditions encountered clinically. Three days following this operation, a second operation is performed. At that time a split-thickness graft of skin is taken from the abdominal wall.

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

PROJECT DESCRIPTION (Continued)

Methods Employed: (Cont'd)

The common duct region is exposed and a portion of the common duct proximal and distal to the area of previous ligation is excised in order to create a defect that is at least 2½ cm. in length. The skin graft is then used to form a tube over a rubber T-tube, and the T-tube is then introduced into both ends of the common duct and an accurate anastomosis between the common duct and skin graft is performed. After the graft is sutured in place, a tab of omentum is placed in the region of the graft. The end of the T-tube is tied off, brought out through a stab wound in the right upper quadrant, and then placed in a subcutaneous pocket in the musculature of the right upper quadrant of the abdomen. The skin graft is constructed into a tube in the direction of Langer's lines in one-half of the dogs and is constructed so that the long axis runs perpendicular to Langer's lines in the remainder of the dogs. The dogs were divided into two groups. The T-tube was removed from the dogs in one group at the end of three months and from the dogs in the other group at the end of six months. The dogs have been observed for varying periods of time following the removal of the T-tubes in order to observe them for evidence of bile duct stricture formation. The dogs will be sacrificed, and gross and histological studies of the common ducts will be performed at the time of autopsy.

Major Findings:

The above procedure was carried out successfully in twenty dogs. There was an operative mortality in three dogs; the dogs dying on the 7th, 12th and 28th postoperative days respectively. There was evidence of leak at the anastomosis site in each of these dogs. Of the twenty dogs in which the procedure was carried out successfully, sixteen of them are living at the present time. Four dogs have died, and these dogs had evidence of constriction at the site of the skin graft in the common duct. It is believed that the cause of death in two of these dogs was distemper.

Significance to Cancer Research:

The repair of common bile duct defects is one of the most difficult problems in surgery today. This is evidenced by the multitude of experimental and operative procedures that have been employed. There is general agreement that it is advantageous to preserve the sphincter of Oddi in reconstruction of the biliary tract. For this reason it would be more preferable to repair common bile ducts by means of a successful transplantation of tissue to bridge existing defects than to effect repair by means of Roux-en-Y procedures that are advocated today. The proposed method of reconstruction of the common

C-10-771
SERIAL NUMBER

Significance to Cancer Research: (cont'd)

bile duct will allow preservation of the sphincter of Oddi and therefore will be of value as a new surgical technique. If successful, this method will be recommended to surgeons for clinical application.

Proposed Course of Project:

After suitable periods of observation, the remaining dogs will be sacrificed and conclusions drawn from this study with regard to the feasibility of this method of common duct repair.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-771
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$2,430	$960	$3,390	1/2	0	1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	0	1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16 and 17

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-7720
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Determination of the Frequency of Recurrence of Chromogen in the Urine of Patients in the Pre- and Postoperative Periods and on Various Types of Diets.
PROJECT TITLE

7. Dr. James R. Jude
PRINCIPAL INVESTIGATOR

8. Dr. Peter Olch and Dr. Peter H'Doubler
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

This project is part of the studies of Dr. W. C. Hueper on "The Possible Relation of Color Reactions with Ortho-Amino-Naphthol Derivatives in Urine to Tryptophane Metabolites". (Project No. OADR-202)

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

In the study of the possible cancer hazards associated with the dyes Yellow AB and Yellow OB, it has been suggested that the metabolism of these compounds produces B-naphthylamine or 2-amino-1-naphthol. In studies regarding the dye metabolism, a color reaction has been produced when the 2-amino-1-naphthol is oxidized under alkaline conditions. In preliminary studies it was noted that in 100 urine samples collected from humans and from dogs, no color reaction was observed. However, in the early clinical studies, three patients were selected to receive Yellow AB. One of these patients showed chromogen in the urine prior to the administration of the dye. It was noted that this patient's diet consisted

(Use reverse and additional pages, if necessary)

C-10-772C
SERIAL NUMBER

PROJECT DESCRIPTION (Continued)

Objectives: (cont'd)

of the high protein preparation, Sustagen, administered by stomach tube. This same color reaction was noted in a second patient receiving the high protein diet but who had not received any dye. It was suspected that in the two patients receiving the high protein diet, the source of the aromatic amine metabolites was most likely from tryptophane. It is the plan of this study to determine the frequency with which substances interferring with the chromogen color test appear in a routine randomly selected group as well as patients that would receive special diets or medications as would occur in the normal management of cancer patients on a surgery ward.

Methods Employed:

Urine from patients on the surgery floor will be collected, as well as urines from some of the patients under radiation therapy. The urines will be saved, and those showing a positive chromogen test will be preserved for further chemical identification by the chemists with Dr. Hueper's group. Those with negative reaction will not be saved. The examination of the urine consists in hydrolysis by boiling the urine with concentrated hydrochloric acid, followed by neutralization with ammonia, appropriate extraction, and when color is observed, the quantitative determination of the substance producing the color by the use of a colorimeter.

Patient Material:

Patient material in this project will be obtained from patients hospitalized on 10 East and on other services in the Cancer Institute.

Major Findings:

The project is just underway. No findings are to be reported.

Significance to Cancer Research:

The use of food dyes throughout the world raises the question of the possibility of this being one of possible carcinogens which, if true, would result in the availability of a real cancer preventive method.

Proposed Course of Project:

It is proposed that during this next six to eight months urine from about 100 patients could be analyzed and this project could be completed by then.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-7720
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	0	0	0	0	0	0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	0	0	0

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. C-10-773
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Surgery Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Heterotransplantation of Tumor in Endocrine-Deficient Animals.
PROJECT TITLE

7. Dr. William E. Schatten
PRINCIPAL INVESTIGATOR

8. Dr. Delbert M. Bergenstal and Dr. William Kramer
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

To evaluate the effect of vitamin deficient states on the alteration of host resistance to the heterotransplantation of tumor tissue.

Methods Employed:

Hypophysectomized, thyroidectomized, ovariectomized, adrenalectomized rats and rats receiving a sham operation are used to evaluate the effect of endocrine deficiencies on host resistance. Sarcoma-37, a mouse tumor, is diluted and inoculated intraperitoneally into rats in each of the above-mentioned groups. Four days following inoculation, the animals

(Use reverse and additional pages, if necessary)

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SERIAL NUMBER

Methods Employed: (cont'd)

are sacrificed and the presence or absence of tumor ascertained. The primary criterion is "take" of tumor. Histologic sections are made of many of the tumors and the ascitic fluid that is present in many of the rats is smeared and stained for cells. Cell counts of the tumor to be inoculated is done before each inoculation.

Hypophysectomized rats are treated with growth hormone, tri-iodo-thyroine, or thyroid-stimulating hormone and S-37 tumor inoculated into these rats in order to see if replacement therapy can affect the "take" of tumors.

Major Findings:

It has been found that heterotransplantation of S-37 is successful in a significantly higher percentage of times when the tumor is inoculated into hypophysectomized rats than when it is inoculated into litter-mate normal controls or rats that have had adrenalectomies, ovariectomies, or sham operations. Heterotrans-plantation is also more successful in rats that are thyroidectomized with radioactive iodine. This portion of the work is being done with Dr. Delbert Bergenstal.

It has been found in both hypothyroid and hypophysectomized rats that the increased incidence of successful tumor "take" follow-ing heterotransplantation can be affected so that the incidence of "takes" is the same as in normal rats when tri-iodo-thyroine is administered prior to inoculation of tumor.

Significance to Cancer Research:

The results of this study show the effect of endocrine control on host resistance. If host resistance could be altered effectively, methods that affect such alteration might well be of value in enhancing oncolytic effects of viruses and other agents following administration to patients with carcinoma. If alteration could be effective to a greater extent, homotransplantation of tissues might be possible. It is also possible that human tumors might be heterotransplanted to laboratory animals if the immune mechanism could be sufficiently altered and this would provide a method for studying individual tumors of patients in laboratory animals.

Proposed Course of Project:

The above-mentioned project is almost completed. Results will be analyzed and conclusions drawn.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. C-10-773
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	0	0	0	0	0	0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	0	0	0

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

No entries for 15, 16, and 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-11-800(C)
SERIAL NUMBER

2. N.C.I.
INSTITUTE OR DIVISION

3. Endocrinology Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Endocrinology
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Endocrine Aspects of the Progression and Therapy of Cancer of the Breast in Women and Men
PROJECT TITLE

7. Roy Hertz, D. M. Bergenstal, and M. C. Li
PRINCIPAL INVESTIGATOR

8. H. A. Lubs, H. J. Levine, B. R. Landau, F. G. Dhyse, S. J. Jackson, F. Durrett, and W. L. Logan (J... Pittman and H. Altman up to 7/1, ...
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: 1. To extend and intensify detailed clinical observati... of the natural course of cancer of the breast in men and women in order to better appraise the effectiveness of various methods of therapy and learn more concerning the etiology and pathogenesis of the disease.

2. To improve upon existing forms of hormonal therapy for palli... tion of advanced breast cancer and to elucidate the mechanisms involved.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

NCI-11-800(C)

Methods employed: 1. The acceptance of complete clinical responsibility for the further management of referred patients with proven diagnosis of cancer of the breast and the application to their problems of all available modes of therapy.

2. The exploration of new modes of hormonal therapy for palliation.

3. The detailed analysis of endocrine and metabolic factors operating in relation to the genesis and course of the disease in these patients up to the time of their demise.

4. Careful appraisal of pathological specimens obtained at biopsy or autopsy in collaboration with Pathology staff.

Major findings - 12/1/55-12/1/56:

1. The total number of cases of breast cancer which have come under our observation now approximate 300. These patients have all been studied with varying degrees of intensity, but they all have contributed to the general clinical background and teaching functions of the group. Thus, a constant liaison is maintained with local physicians and medical institutions.

2. Massive parenteral estrogen therapy in water-soluble form has now become an accepted form of estrogen administration for breast cancer therapy. Moreover, the toxicological data obtained from these clinical studies has facilitated the development of similar dosage regimens for various gynecological disorders. Consequently, there are now three brands of commercially available parenteral estrogen preparations for clinical use. Whereas, our own studies still do not permit a clear demonstration of any superiority of this form of estrogen therapy over more conventional regimens, we continue to find it a practicable and effective procedure which has now been adapted in other services, particularly in relation to the treatment of menorrhagia, prostate cancer, and breast cancer.

3. Limited exploration of the feasibility of massive intravenous androgen therapy indicate that with available chemical forms of androgens the procedure will probably not be feasible. However, in view of the development of water soluble hemisuccinate of hydrocortisone for practical clinical use it is still possible that a comparable form of water soluble androgen may be developed. This has been pursued with industrial cooperation, but thus far with no practical solution to the problem.

Major findings (cont'd)

4. In collaboration with the Neurosurgical Service of NINDB a comprehensive program for the study of hypophysectomy in breast cancer and related malignancies has been initiated. All of the clinical and organizational problems of preoperative and post-operative care have been worked out. Excellent cooperation with theAnesthesia department has been developed. Quantitative endocrine and metabolic criteria for the completeness of hypophysectomy have been developed. These include: radio-iodine uptake by the thyroid, protein bound iodine and cholesterol determination on the plasma, plasma and urinary corticoid determinations, urinary ketosteroid and gonadotropin determination, glucose tolerance and insulin sensitivity tests and basal metabolic rate. In addition, active research is under way to develop even more sensitive and precise biological and biochemical indices of hypophyseal loss. These include studies on the possible direct enhancement of endogenous ACTH effectiveness by posterior pituitary extract and assay of the urine for residual melanocyte-stimulating hormone by the frog-skin test.

Clinical observations on the therapeutic role of hypophysectomy in breast cancer are as yet too limited for evaluation. However, sufficient response has been observed in a few instances to merit extended study of this remarkable procedure. In any case, the clinical feasibility of the procedure is clearly demonstrated even in advanced cases. These studies should provide definitive information concerning the role of hypophyseal factors in malignancy as well as in many other normal and abnormal processes.

5. The dietetic, clinical and laboratory procedures for complete balance study of patients with respect to sodium, potassium, calcium, phosphorus and nitrogens have been completely worked out. These studies have been properly integrated with more specialized balance studies of riboflavin, niacin, pantothenate, folic, pyridoxine, biotin, and amino acids. In addition, correlated studies on fat and polysaccharide metabolism have been undertaken. Balance procedures of this type have thus far provided the following observations:

(a) A hypophysectomized patient given highly purified monkey growth hormone exhibited marked nitrogen retention despite a distinct elevation of radioiodine uptake by the thyroid which would be expected to produce a catabolic effect. Growth hormone preparations from other species have failed to induce anabolic effects in the human. The availability of such a

Major findings (cont'd)

potent growth hormone preparation for clinical studies opens a wide field of study of the role of this hormone in normal growth and in malignancy in the human subject.

(b) A complete characterization of the metabolic effect of Δ_1 Fluorohydrocortisone in 6 patients indicated that its extreme sodium retaining and potassium diuretic effect makes it a somewhat hazardous agent for the sole maintenance of adrenalectomized patients for more than a few day's time. Thus, the expectation that its enhanced corticoid potency would permit the maintenance of adrenalectomized breast cancer patients on a regimen which would provide less steroid as a potential source of exogenous estrogen has not been fulfilled.

(c) Four patients have been shown to exhibit a profound anabolic response to oral administration of 17-ethyl 19-nortestosterone. (See below for additional therapeutic studies with this compound).

(d) No significant metabolic effect of 17-ethinyl-19 nortestosterone, a highly potent oral progestational compound was observed in two patients. (See below for additional therapeutic studies with this compound). This indicates that even such massive doses of 200 mgm. daily may be given for therapeutic trial without hazard of serious derangement of nitrogen or electrolyte balance.

(e) In 8 patients in whom steroid-induced nitrogen retention has been observed there was an associated retention of pantothenate. This pantothenate retention followed the nitrogen retention both temporally and quantitatively and appears to reflect some role of pantothenate in the response. In any case, this represents the first demonstration that pantothenate is of critical metabolic importance in the human subject. It is noteworthy that this pantothenate effect is associated with no significant change in the balance of the other elements of the B-complex.

(f) Two acromegalic patients have been studied with respect to the effect of orinase upon their nitrogen and carbohydrate balance. Restoration of both nitrogen and carbohydrate balance toward normal was observed following orinase in both these subjects. These findings indicate that the diabetogenic action of

Major findings (cont'd)

the adenomatous pituitary is in effect neutralized by the action of orinase. Hence no significant difference between acromegalic diabetes and other forms of this disease is demonstrable with orinase administration. Similarly, the hypoglycemic action of orinase was found to be unaltered in patients with hyperthyroidism or hypothyroidism, before adrenalectomy or hypophysectomy, or during ACTH administration.

6. Evaluation of 4 new steroid compounds in the palliation of metastatic breast cancer has been undertaken. (a) Ten patients treated with Δ1 Fluorohydrocortisone showed little therapeutic advantage over hydrocortisone and exhibited sufficient electrolyte disturbance that maintenance therapy with this compound despite excessive potassium ingestion proved to be hazardous and impractical.

(b) Studies with 9-α-fluoro 11-hydroxy 17-methyl testosterone appeared indicated since this compound is 5 to 10 times as active as methyl testosterone when given orally. Fifteen patients have been observed on doses ranging from 20 to 60 mgm. daily. Preliminary observations indicate that the potent oral androgen will produce regression of both soft tissue and osseous metastases. Its relative androgenicity is not yet fully determined but it is already clear that all of the undesirable side effects of hirsutism, voice change, and clitoral hypertrophy are observable in patients receiving this medication. Occasional mild nausea and anorexia have also been observed.

(c) 17-ethinyl 19-nortestosterone is a compound which has been shown to be a highly active oral progestational agent in women. Twelve breast cancer patients have thus far been observed while on doses ranging from 40 mgm. daily. Distinct therapeutic effects in terms of reduction of hypercalcemia and hypercalcinuria and regression of soft-tissue lesions have been observed. Uterine bleeding has invariably followed the withdrawal of this compound and mild nausea and anorexia has also been reported. It remains to be determined whether this agent exerts its effects by virtue of its essential progestational action or by virtue of the estrogenic or possibly androgenic effects of its metabolites.

(d) 17-ethyl 19-nortestosterone is a potent oral anabolic agent which is reputed to have proportionally less androgenic effect. Ten patients have been observed under treatment with this agent

Major findings (cont'd)

and the results appear to be essentially comparable to those obtained with other androgens.

Significance to cancer research:

The direct pertinence of the foregoing studies to the grave problem of advanced or persistent breast cancer is too obvious to require discussion.

Proposed course of project:

The further course of these studies are to be essentially along the lines outlined above. Emphasis is to be placed on balance studies and the evaluation of hypophysectomy in breast cancer. Further evaluation of new steroids and related compounds is to be made.

Please note:

Project NCI-810(C) was discontinued at the start of this calendar year. The use of radioactive iodine for thyroid function studies has been incorporated into Project NCI-800(C).

Project NCI-807(C) has been discontinued with the resignation of the principal investigator. The personnel assigned and this area of work are absorbed into Projects NCI-800(C), 803(C) and 808(C).

Project NCI-805(C) has been discontinued and this phase of our work is now included in Project NCI-800(C).

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-11-800(C)
SERIAL NUMBER

12. BUDGET DATA:

ESTIMATED OBLIGATIONS			MAN YEARS		
DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
$78,570	$141,320	$219,890	5	3 2/3	8 2/3

FY'57

BUDGETED POSITIONS			PATIENT DAYS
PROF	OTHER	TOTAL	42 pts. 1642 pt. days
5	4 2/3	9 2/3	

FY'57

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

The Neurological Service of the NINDB is cooperating in the hypophysectomy program.

(Use reverse and additional pages, if necessary)

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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C. Honors, Awards and Publications 15. NCI-11-800(C)
SERIAL NUMBER

16. LIST OF PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. R. Hertz: Physiologic Effects of Androgens and Estrogens in Man, Am. J. Med. 21: 671, 1956 (as part of symposium on Hormones and Cancer).

17. Honors:

Dr. D. M. Bergenstal, 1. Appointed Member of the Clinical Panel of the National Cancer Chemotherapy Center and liaison member to the Endocrine Panel.

2. Invited to lecture on "Functions of the Pituitary in Man" American Academy of Neurological Surgery, Phoenix, Arizona, November 8, 1956.

3. Invited to lecture on "Use and Abuse of Adrenal Steroid Therapy" Southern Medical Association, Washington, D. C.,November 13, 1956.

4. Invited to lecture on "Hypophysectomy for Control of Metastatic Carcinoma", Washington Clinic, October 9, 1956.

5. Presided over session of Laurentian Hormone Conference, September 1956.

Dr. R. Hertz: 1. Appointed Chairman of Advisory Committee on Therapy of the American Cancer Society, September 1956.

2. Elected to Council of The Endocrine Society, June 1956.

3. Reappointed member of Editorial Board of ENDOCRINOLOGY.

4. Invited to lecture before American Association of Military Surgeons, November 1956. Title of lecture: "Chemotherapeutic Studies of Hormone-Producing Tumors of Adrenal and Trophoblastic Origin" to be published in Military Medicine.

5. Invited to lecture before Scientific Session of American Cancer Society, New York, October 1956. Title of paper:

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NCI-11-800(C)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Honors (cont'd) Dr. Roy Hertz -

"Attempts to Suppress Adrenal Function by Medical Means."

6. Invited to participate in Third National Cancer Congress, Detroit, Michigan, June 1956.

7. Presided over session of Endocrine Society, June 1956.

8. Presided over session of Laurentian Hormone Conference, September 1956.

9. Lectured at Yeshiva Medical School, New York, October 1956. Title of lecture: "Endocrinologic Studies on Hormone-Producing Tumors."

10. Invited to speak to the Peninsula Medical Academy, Langley AFB, Virginia, March 1956. Title of paper: Some Instructive Endocrinopaties."

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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-11-801(C)
SERIAL NUMBER

2. N.C.I.
INSTITUTE OR DIVISION

3. Endocrinology Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Endocrinology Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Endocrine Aspects of Cancer of the Prostate in Man
PROJECT TITLE

7. D. M. Bergenstal, R. Hertz, and M. C. Li
PRINCIPAL INVESTIGATOR

8. Lois Hallman, Herbert Lubs, Herbert Levine (J. A. Pittman up to 7/1/56)
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: 1. To improve non-surgical therapy of cancer of the prostate.
2. To increase understanding of endocrine factors involved in genesis and course of prostate cancer.
3. To gain basic knowledge of the metabolic action of certain steroids in man.

Methods Employed: Little progress on this project is to be reported because of lack of appropriate cases.

Three patients were given 19 nor-ethinyl testosterone in doses of 20 to 200 mgm. daily by mouth. Little effect was noted on pain, acid phosphatase titre or general clinical course of the patient except

(Use reverse and additional pages, if necessary)

Methods Employed (cont'd)

for apparent exacerbation on 2 occasions in one patient.

One patient was treated with massive methotrexate therapy with a marked but transient drop in acid phosphatase titre but with associated evidence of bone marrow depression and renal damage both of which were reversible. No apparent therapeutic effect on the subsequent course of his disease was noted.

Significance to Cancer Research: The high frequency of prostatic carcinoma in man and initial inroads upon it by hormonal therapy indicate the pertinence of this study to cancer research.

Proposed Course of Project: The further development of this project will have to be determined by the case material available. Emphasis will be upon hypophysectomy and upon evaluation of newer steroids.

Part C: Honors, Awards and Publications: No entries.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-11-801(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$28,990	$38,730	$67,720	3 1/2		3 1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	15 pts. - 450 pt. days
FY'57	3	1	4	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-802(C)
SERIAL NUMBER

2. N.C.I.
INSTITUTE OR DIVISION

3. Endocrinology Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Endocrinology Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Preoperative Hormonal Therapy of Cancer of Cervix
PROJECT TITLE

7. Roy Hertz, John H. Waite, and Robert Smith
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine the value of steroid therapy as a palliative measure in advanced cancer of the cervix.

Methods employed: Patients who are considered amenable to definitive surgical therapy are pre-treated for 4 to 6 weeks with massive doses of various steroid hormones singly and in combination and then are operated upon. Their progress is evaluated by frequent pelvic examination, serial biopsies and smears and by histopathological study of the surgical specimens removed. Pyelograms and barium enemata are also employed to evaluate changes in the pelvic viscera. The amount of vaginal bleeding is checked from day to day. The patient's general clinical status with respect to weight,

(Use reverse and additional pages, if necessary)

Methods employed (cont'd)

appetite, blood status, and pain are also closely observed.

Major findings: This project has not been advanced during this year due to the justified pre-occupation of the Surgical Staff with the virus approach.

Significance to cancer research: Palliation of advanced cervical carcinoma is urgently needed in view of the over-all 5 year survival rate with this disease (40-50%).

Proposed course of project: The further course of these studies will depend upon the interests of the surgical staff which is at the moment justifiably concentrated on the virus approach to cervical cancer.

Part C. Honors, Awards and Publications: No entries.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-802(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	0	0	0	0	0	0

Not activated this year.

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	0	0	0

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Surgery Branch, NCI.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-12-803(C)
SERIAL NUMBER

2. N.C.I.
INSTITUTE OR DIVISION

3. Endocrinology Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Endocrinology Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The Nature of Hormone-Producing Tumors of Pituitary, Adrenal, Ovary, Testis, Pancreas, Parathyroid and Chorion; Abnormalities of Somatic Development and Growth.
PROJECT TITLE

7. Roy Hertz, D. M. Bergenstal, and M. C. Li
PRINCIPAL INVESTIGATOR

8. Herbert A. Lubs, Herbert J. Levine, B. R. Landau, W. W. Tullner, Donald Spencer, Silas Jackson, Foster Burnett, Willie Logan (J. A. Pittman, M. M. Graff and S. R. Fand to July 1, 1956)
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: 1. To learn more about the basic metabolic phenomena involved in the process whereby neoplasms in hormone-producing organs produce highly excessive amounts of hormone, resulting in such syndromes as Cushing's Disease, Acromegaly, Hyperparathyroidism, Hyperadrenalism, and similar endocrinopaties.

2. To maintain in tissue culture hormone-producing tissues and to ascertain the factors involved in quantitative hormone production in vitro.

3. To assess the capacity of new drugs to alter the course and hormonal activity of hormone-producing tumors.

(Use reverse and additional pages, if necessary)

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Methods employed: Hormone producing tumors from patients with one of the above-named syndromes are obtained at surgery and carried in tissue culture. The supernatant fluid from the cultures are tested for hormonal activity by appropriate bioassay methods and the quantity recovered compared with the content of a piece of tissue exactly equivalent to that originally placed in culture.

Patients presenting such syndromes are completely characterized endocrinologically and metabolically before and after therapy in order to ascertain the relative effects of various forms of therapy.

Major findings: 1. Normal pregnancy is characterized among other distinctive metabolic findings by a high urinary gonadotropin titre associated with a marked histidinuria. The latter feature has now been shown to be consistently lacking in both male and female patients suffering from gonadotropin-producing tumors. During the past five years a total of 14 cases of hydatid mole, 7 cases with choriocarcinoma or chorioadenoma of the uterus, and 5 cases of choriocarcinoma of the testis have all without exception shown a lack of histidinuria despite a high urinary gonadotropin titre. We feel that we now have a firm basis for a diagnostic test permitting the differentiation of normal pregnancy from choriocarcinoma or chorioadenoma.

2. An unconventional regimen for the administration of intensive anti-folic or anti-purine therapy has been employed in 6 cases of gonadotropin-producing tumors, namely 2 cases of choriocarcinoma of the uterus, 1 case of chorioadenoma, 3 cases of embryonal carcinoma of the testis with apparent functional chorionic elements. These patients have been followed by x-ray, gonadotropin titre in urine and plasma, and by such general clinical indices as blood count, body weight, appetite, and changes in skin and mucous membranes. The data support the conclusion that (a) short intensive courses of methotrexate or 6 mercaptopurine approximately 6 times greater than conventional dosage are well tolerated provided adequate spacing of courses for full recovery from possible marrow and mucous-membrane injury is permitted. The management of each case is highly individualized but the courses have averaged 5 days in length and the intervals have ranged from 7 to 10 days.

(b) The urinary gonadotropin titre may be acutely depressed by any given course of therapy and will return in a few days either to its prior level or to substantially lower levels. In 4 of

Major findings (Cont'd)

these patients the titres have ultimately come down to essentially normal levels and have remained at such low levels for periods of 2, 7, 8, and 13 months respectively.

(c) The vagaries of the clinical behavior of such tumors demand utmost conservatism in the evaluation of the therapeutic effects obtained. Nevertheless, the consistently favorable radiological and clinical course in the 4 patients who have thus far been under sufficiently prolonged therapy for evaluation when correlated with the remarkable reduction in urinary gonadotropin titre in these cases indicates that some degree of reproduceable suppression of these tumors has been obtained.

3. Further attempts to transplant human hormone-producing tumor tissue to cortisone-treated irradiated rats have been pursued. Transplants of such hydatid mole cysts spread into thin sheets and placed under the rat's kidney capsule have persisted in endocrinologically and histologically preserved form for 3 weeks. However, second generation transplants consistently underwent rapid necrosis with the apparent discharge of their residual hormone content leading to hypertrophy of the host's ovaries and uterus. Thus, the functional survival of hydatid tumor tissue in the rat for limited periods of time has been demonstrated, but its actual growth has consistently failed.

Similar failure has been experienced in 3 additional cases of adrenal carcinoma notwithstanding attempted transplantation to the adrenal cortex of ACTH treated rats.

4. Two additional gonadotropin-producing cultures of hydatid mole and choriocarcinoma have been initiated and are now surviving several months in the first generation. A culture derived from an islet cell tumor has been maintained for several months. Assay of the fluids from this culture have been deferred pending the development of more sensitive methods for insulin assay by the in vitro rat diaphragm technique.

5. Extensive clinical and metabolic studies on the effect of Amphenone upon human adrenal and thyroid function in patients with normal or with excessive adrenal activity have now been performed. In all, 14 cases of adrenal carcinoma have been studied in our own and in collaborating laboratories. Additional observations on 3 cases of adrenal hyperplasia have been carried out.

Major findings (cont'd)

With the aid of C_{14} tagged hydrocortisone and corticosterone, the suppression of synthesis of both substances under the influence of Amphenone is conclusively demonstrated. Clinical manifestations of hyperadrenocorticism such as hypertension, diabetes, and mental abberations have been repeatedly reversed by Amphenone administration. Unfortunately, clinical and histological observations thus far reveal no apparent effect of Amphenone on the progress of the neoplastic process. Moreover, the toxic side-effects of Amphenone are such as to limit its usefulness to extreme clinical situations. Still, a beginning has been made toward the chemical control of adrenal cortical function.

6. A very useful body of clinical material presenting a great variety of abnormalities of somatic developments is under constant observation. This includes 8 cases of ovarian agenesis, 1 case of true hermaphroditism, 2 cases of male pseudohermaphroditism, and 6 cases of sexual precocity.

7. Metabolic studies on acromegaly have shown that the diabetogenic effect of the adenomatous pituitary is readily counteracted by orinase, one of the new anti-diabetic sulfonyl urea derivatives. This has been demonstrated in two patients on complete balance study. These findings aid in the characterization of the nature of the diabetes associated with such pituitary tumors.

8. Improved methodology for the determination of urinary corticoids, urinary ketosteroids, and plasma corticoids has been developed. These determinations have been checked by other laboratories and by the use of isotope dilution methods. Mensuration of these steroid factors in plasma and urine is now on a more secure biochemical basis.

Significance to cancer research: Further understanding of the conditions permitting the excessive hormonal production of tumors of endocrine origin is basic to our understanding of the metabolism of neoplastic tissue generally. In addition, further analysis of abnormalities in somatic growth and development should provide data pertinent to the problems of tissue growth generally.

Proposed course of project: Further elaboration of these studies with major emphasis on biochemical and pharmacological control of hormone-producing tumors and anomalous tissue growth is projected.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-12-803(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$74,800	$75,580	$153,380	5 1/2	4	9 1/2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	30 pts. - 913 pt. days
FY'57	5 1/2	5	10 1/2	

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Dr. Earle's Tissue Culture Unit collaborates in this program.

(Use reverse and additional pages, if necessary)

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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C. Honors, Awards and Publications 15. NCI-12-803(C)
SERIAL NUMBER

16. LIST OF PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. M. C. Li, R. Hertz, and D. B. Spencer: Effect of Methotrexate Therapy Upon Choriocarcinoma and Chorioadenoma, Proc. Soc. Exper. Biol. & Med. 93: 361-366, 1956.

2. R. Hertz, A. E. Renold, W. J. Reddy, J. A. Pittman, M. M. Graff, and G. W. Thorn: Pharmacological Alteration of Adrenalcortical Function in Man: Amphenone and Related Compounds, Third National Cancer Conference, Trans. Assoc. American Physicians for 1956.

3. G. W. Thorn, A. E. Renold, A. Goldfien, D. H. Nelson, W. J. Reddy and R. Hertz: Inhibition of Corticosteroid Secretion by Amphenone in a Patient with Adrenocortical Carcinoma, N. Eng. J. Med. 254: 547: 1956.

4. R. Hertz, J. H. Waite, and L. B. Thomas: Progestational Effectiveness of 19-nor-ethinyl-testosterone by Oral Route in Women, Proc. Soc. Exper. Biol. & Med. 91: 418, 1956.

5. R. Hertz, J. A. Pittman and M. M. Graff: Amphenone: Toxicity and Effects on Adrenal and Thyroid Function in Man, J. Clin. Endocrinol. & Metab. 16: 705, 1956.

6. J. A. Pittman and B. B. Westfall: Spectrophotometric Estimation of Amphenone in Biological Material and its Absorption and Excretion in Man, J. Pharm. & Exper. Therap., Dec. 1956.

7. R. Hertz: Attempts to Suppress Adrenal Function by Medical Means, presented at the Scientific Session of the American Cancer Society in New York, October 1956.

17. Honors: R. Hertz. 1. Invited to present paper "Pharmacological alteration of adrenalcortical function in man: Amphenone and related compounds" before American Association of Physicians - May 1956.

M. C. Li. 1. Invited to present seminar at Memorial Hospital, New York, September 19, 1956.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-11-804(C)
SERIAL NUMBER

2. N.C.I.
INSTITUTE OR DIVISION

3. Endocrinology Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Endocrinology Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Biotin and the Avidin-Biotin Complex in Relation to Normal and Neoplastic Tissue Growth
PROJECT TITLE

7. R. Hertz, F. G. Dhyse, and Phillip Rayford
PRINCIPAL INVESTIGATOR

8. Lloyd Law and Virginia J. Evans
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: 1. To ascertain the role of biotin, a dietary trace factor, in the growth of normal tissue and in the development of neoplasms in animals and man.

2. To learn the normal metabolism of biotin in man and to produce biotin deficiency in cancer patients administering low biotin diets plus concentrates of avidin prepared from egg-white.

3. To develop methods for the cheap and practical preparation of large amounts of avidin for therapeutic trial in selected cancer cases.

(Use reverse and additional pages, if necessary)

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Methods employed: 1. Standard methods of protein fractionation of egg-white are employed to prepare potent avidin concentrates which are assayed for growth suppressing effect on a biotin requiring yeast organism.

2. Biotin deficiency is produced in mice by feeding egg-white and avidin concentrates and the effect of this deficiency on such standardized tumors as transplantable leukemia is determined in collaboration with Dr. Lloyd Law.

3. Patients are carried on diets of known and constant biotin content and the urinary biotin excretion is followed microbiologically. The effects of egg-white feeding and avidin feeding are observed upon the patient's clinical nutritional status and upon the urinary excretion of biotin.

Major findings: 1. In anticipation of clinical studies, additional supplies of avidin have been prepared in collaboration with a commercial laboratory.

2. A substantial but not complete biotin deficiency was produced in mice which were subsequently challenged with leukaemia. The deficient animals appeared to support the tumor process as well as the biotin-supplemented controls.

3. The coliform organisms of the bowel of the rat synthesize moderate amounts of an avidin-uncombinable form of biotin. The feeding of an anti-biotic called actithiazic acid enhances this synthesis. This anti-biotic was previously shown to interfere with the growth of a biotin-dependent strain of tubercle bacilli. It appears from our studies to play some as yet enigmatic role in biotin metabolism presumably by means of its action on the coliform organisms resident in the bowel.

4. A number of biotin analogues have been tested for their potential anti-biotin activity in the rat. Notwithstanding the anti-biotin action of several of these compounds in microbiological tests we have not been able to demonstrate an anti-biotin effect with any of these in the rat. Thus, the only available agent for the production of biotin deficiency is still avidin.

5. Tissue culture studies have shown that in a synthetic medium, the growth of Strain L fibroblasts is inhibited by addition of avidin to the medium and this inhibition is reversed by excess biotin. Thus a mammalian cell is shown for the first time to require biotin when grown in vitro. This system should prove notable

Major findings (cont'd)

for the testing of biotin antagonists.

Significance to cancer research: Numerous experimental animal studies show a certain dyes in biotin deficient animals. Induced biotin deficiency may parallel induced folic acid deficiency in depressing certain specific tumor growths with at least a narrow margin of safety with respect to the tissues of the host. At least this same principle ought to be tested out in relation to biotin.

Proposed course of project: These studies are to be pursued at both a clinical and laboratory level. Additional biotin analogues will be sought out and tested in microbiol and animal systems. Further effort will be made to simplify the methods of avidin concentration from egg-white employing chromatographic adsorption of specific protein fractions.

Part C. Honors, Awards and Publications: No entries.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-11-804(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$13,310	$5,290	$18,600	1/3	1 2/3	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2	2 1/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Dr. Lloyd Law, Leukemia Studies Section, Laboratory of Biology, NCI.
Dr. Virginia Evans, Tissue Culture Unit, Laboratory of Biology, NCI.

(Use reverse and additional pages, if necessary)

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(Attachment I)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-13-808(C)
SERIAL NUMBER

2. N.C.I.
INSTITUTE OR DIVISION

3. Endocrinology Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Endocrinology Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Endocrine Factors Governing Hormone-Induced Tissue Growth and Tumor Formation in Animals; Bioassay of Blood and Urine from Cancer Patients
PROJECT TITLE

7. Roy Hertz, William W. Tullner, and D. M. Bergenstal
PRINCIPAL INVESTIGATOR

8. D. B. Spencer, Oscar Vilar, Lois Hallman, S. Jackson, F. G. Dhyse, and S. Siegel
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: 1. To gain basic information concerning endocrinological and nutritional factors governing hormonally conditioned growth and involution in organs such as the uterus, breast and prostate which are the frequent seat of hormone-sensitive neoplasms.

2. To develop and improve bioassay methods for the quantitation of hormones in the blood and urine of patients with cancer who are either receiving various forms of hormonal therapy or who present unusual hormonal patterns.

(Use reverse and additional pages, if necessary)

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Objectives (cont'd)

3. To determine the biological properties of steroid and related compounds in terms of their potential usefulness in cancer therapy.

Methods employed: Standard methods of biological study are employed. These include: 1) the maintenance of animals of various species on specified hormonal or nutritional regimens; 2) daily observations of physical and behavioral changes in such animals; 3) complete autopsy of test animals with detailed weighing and chemical analysis and histochemical study of specific organs; 4) surgical alteration of the endocrine status of animals by gonadectomy, adrenalectomy, hypophysectomy, thyroidectomy, parabiosis, hysterectomy and other operative procedures.

Major findings: 1. Previous observations in this laboratory have demonstrated that the pre-puberal differentiation of the rat and rabbit ovary is pituitary-independent. This observation has been confirmed in the rat by the demonstration in tissue culture in synthetic medium of the early differentiation of the day-old ovary. Also, it has been demonstrated that extensive hypothalamic damage sufficient to impair normal pituitary gonadotropin function does not alter this early pre-puberal process of gonadal development. These studies were suggested by the clinical occurrence of cases of sexual precocity in patients with hypothalamic tumors.

2. Collaboration with Dr. Max Ehrenstein of the University of Pennsylvania has permitted the comparative assay of the two stereo-isomers of 19-norprogesterone. They were found to have equal potency, thus establishing that an unnatural configuration at carbon-17 does not impair biological potency.

Long-term studies with the halogenated corticoids shown to have progestational activity have failed to reveal the expected metaplastic changes in the endometrium of the rabbit. Instead, profound atrophy was induced, suggesting a secondary negative reaction to the initial growth stimulus in the endometrium.

3. Further efforts have been applied to refine the bio-assay of growth hormone in relation to the effect of the substance on the uptake of radioactive sulfur by various cartillaginous structures in the body. Thus insulin and triiodothyronine both synergize with growth hormone in promoting S-35 uptake and increase the sensitivity of the response by about 20%. Moreover,

Major findings (cont'd)

locally applied growth hormone is seen to exert its action directly upon the treated cartillagenous tissue without concommittant effect on contra-lateral control tissues. Also, timed studies indicate that Sulfur and growth hormone must be delivered simultaneously to the site of action, thus indicating no effect of growth hormone in mobilizing pre-existing chondroitin-sulfate. Hydrocortisone inhibition of S-35 uptake by cartillage is reversed by administering growth hormone.

4. The heterologous transplantation of S-37 mouse ascites tumor to the rat is greatly enhanced by hypophysectomy or by radiothyroidectomy. These effects do not depend upon reduced food intake as demonstrated by paired feeding experiments. Hence the pituitary and thyroid as well as the adrenal are involved in heterologous tissue reactions and immune processes. The clinical applicability of these findings to the problem of skin-grafting is being explored.

5. Twenty additional analogues of Amphenone have been assayed in the rat and have been found to be inactive for adrenal or thyroid effect. Additional compounds are now provided from three industrial laboratories for testing. Only in this way will a compound with less toxicity than Amphenone be derived.

6. Studies on the corticoid content of the adrenal vein blood of the dog indicate that chronic administration of D.D.D., like Amphenone, induces a marked reduction in corticoid output even in response to massive ACTH dosage. Such dogs, however, exhibit normal electrolytes, normal blood pressure and lose little weight thus indicating an apparent indifference to the low corticoid output of the adrenal. However, histologically the glomerulosa is seen to be spared from the drastic necrosis which affects the other cortical zones. It is inferred that the resistant glomerulosa protects the animal from chronic adrenal insufficiency.

7. Chemical studies of the dog's adrenal gland in relation to various states of secretory activity indicate that contrary to what is seen in the rat, adrenal activation by ACTH does not deplete either cholesterol or ascorbic acid content nor does it induce any material change in the B-vitamin content of either the adrenal tissue or the venous effluent.

Major findings (cont'd)

8. A potent estrogen antagonist (1-(p-diethylaminoethoxyphenyl)-1-phenyl-2-p-anisylethanol) has been found to neutralize the effect of exogenous estrogen and of endogenous estrogen in the monkey, rat, and mouse. This compound will prove of great practical and theoretical interest as soon as adequate supplies become available. Its essential biological properties were described by an industrial laboratory whose cooperation has permitted our extension of their findings in the monkey and rat.

9. A genetically determined genital tract defect in AXC rats described by Heston and Deringer has been analyzed from an endocrinological point of view. In the male, one testis appears normal whereas the other is markedly atrophic and lacks an associated epididymis, vas deferens, and seminal vesicle although the ipsilateral coagulating gland is normal. Removal of the abnormal testis leads to no involution of the normal seminal vesicle or of the prostate, indicating adequate androgen output from this abnormal testis. Moreover, the atrophic testis undergoes a slow but progressive compensatory hypertrophy so that after one month it has attained approximately two-thirds normal size. The neoplastic potentiality of this abnormal testis will be studied in this tumor-prone strain, it being expected that the compensatory hypertrophy effect may lead to such a change in this congenitally abnormal tissue. Histochemical studies of these tissues are in progress.

In the female of this strain, there frequently occurs a profound hypoplasia of one uterine horn, the contra-lateral horn appearing entirely normal. In such cases pregnancy is frequently seen to consist of one very large fetus in the normal uterine horn, a sort of primitive form of single birth. Massive estrogen administration is totally ineffectual in stimulating the growth of the atrophic horn, suggesting some inherent local resistance to the trophic action of the hormone notwithstanding a completely adequate blood supply. This local refactoriness merits further study.

10. Attempts to produce terratoid tumors in the rabbit by the intra-testicular transplantation of fertilized ova to the testicle of the sire have thus far been unsuccessful. More advanced blastulae are now being investigated.

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Major findings (cont'd)

11. The pregnant guinea pig has been found to have an enormous level of plasma corticoid as determined by chemical means and confirmed by isotope dilution studies. These levels average some 10 times that seen in normal adult female pigs or in adult women. The titre drops shortly after delivery. The possible placental origin of this high corticoid level is being investigated. It is expected that the pregnant guinea pig may prove a good test animal for testing Amphenone-like inhibitors of adrenal function.

12. The fonadotropin assay technique is being improved upon by the development of adsorption and pressure filtration techniques for the concentration of the hormonal activity of the urine. This should expedite the studies on gonadotropin-hormone producing tumors which have created excessive demands upon our bioassay laboratory.

13. Further refinements of one bio-assay technique for estrogen-like activity in human urine have been developed. This will permit the assessment of the effect of hypophysectomy in breast cancer patients upon endogenous estrogen production of ovarian or adrenal origin.

14. The functional adrenal tumor described by Mulay and Eyestone in the Osborne-Mendel rat has been adapted for rapid growth by the Selye air-pouch technique. Massive tumor growth has been obtained in one instance within 6 weeks. Various attempts to transform this tumor into an ascites form have thus far failed but additional trials are in progress. The availability of such a functional tumor for chemotherapeutic trial would be highly advantagenous.

Proposed course of project:

It is expected to continue and extend the foregoing studies along the lines already indicated.

Please Note:

Project NCI-811(C) was terminated in 1954. Some of these activities have been incorporated into Project NCI-808(C).

Project NCI-807(C) has been discontinued and that work has been absorbed by Project NCI-808(C), in part.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-13-808(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$46,140	$12,040	$58,150	3 2/3	3	6 2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	5 pts. - 140 pt. days
FY'57	3 2/3	4	7 2/3	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Dr. Virginia Evans, Tissue Culture Unit, Laboratory of Biology;
Dr. Wm. Schatton, Surgery Branch, NCI.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C. Honors, Awards and Publications 15. NCI-13-808(C)
SERIAL NUMBER

16. LIST OF PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. R. Hertz and W. W. Tullner: Progestational Activity of Halogenated Corticosteroids and Related Compounds in Rabbit and Monkey, Proc. Soc. Exper. Biol. & Med. 91: 76, 1956.

2. W. W. Tullner and R. Hertz: The Effect of 17-α-hydroxy-11-Desoxycorticosterone on Estrogen-Stimulated Chick Oviduct Growth, Endocrinology 58: 282, 1956.

3. W. W. Tullner and R. Hertz: Progestational Activity of 19-Norprogesterone and 19-Norethisterone in the Rhesus Monkey, Proc. Soc. Exper. Biol. & Med. (In press).

4. W. W. Tullner, M. M. Graff, and R. Hertz: Amphenone Inhibition of Adrenal Corticosteroid Output in the Hypophysectomized Dog, Endocrinology 58: 802, 1956.

17. Honors:

Mr. William W. Tullner has passed his written examination for the degree of Doctor of Philosophy at George Washington University, Dept. of Physiology.

Mr. Donald B. Spencer was cited for his demonstrations to visiting Science Teachers.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-12-809(C)
SERIAL NUMBER

2. N.C.I.
INSTITUTE OR DIVISION

3. Endocrinology Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Endocrinology Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. The Preparation of Crude Natural Extracts of Hormonal Activity from Ovary, Testis, Adrenal, and Pituitary and their Biological and Clinical Characterization
PROJECT TITLE

7. Roy Hertz
PRINCIPAL INVESTIGATOR

8. Britton H. Smith and Howard L. Erwin
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To prepare for biological and clinical assay crude preparations of hormonally active tissues in order to determine their effect upon tumors in hormone-sensitive organs. We know the crystalline steroids now so widely used represent only partial elements of the over-all activity of the various endocrine organs and that even optimum mixtures of them do not reproduce the characteristic effect of the total internal secretion of the endocrine gland itself. Such effects may be more profound and more desirable than those obtained by the separate synthetic crystalline factors.

(Use reverse and additional pages, if necessary)

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NCI-12-809(C)

Methods employed: 1. Standard chemical methods of total lipid and protein extraction of freshly prepared tissues to be collected at the slaughterhouse and processed immediately.

2. The application of bioassay techniques to such preparations to determine their endocrine effectiveness and their toxicity.

3. Clinical trial of non-toxic, active materials in suitable patients with endocrine deficiency or cancer of breast, prostate or cervix.

Major findings: 1. Follicular fluid has been collected from pig ovaries in the pre-ovulatory phase of the cycle. Bioassay of freshly preserved material is shown to equal 1 microgram equivalent of estrone per cc. This activity is not of the "impeded estrogen" type described by Huggins et al. for estriol but appears to be more like that of estradiol.

2. Attempts to use the natural estrogen-protein complex of follicular fluid as antigen with the aid of Freund's adjuvant in the rabbit have failed to yield sera with inhibitory action against estradiol when assayed in the immature rat. Further attempts with more concentrated "antigen" preparations are in progress.

3. The allantoic fluid of the pig contains no detectable estrogenic potency.

4. The amniotic fluid possesses about 10% the estrogenic activity of follicular fluid and it is also of the unimpeded type.

Significance to cancer research: The availability of non-toxic concentrates of naturally occurring hormonally active substances or of inhibitory anti-sera capable of neutralizing such endogenous biological effects in the cancer patient would permit therapeutic investigations along lines now neglected as a result of the emphashis on synthetic compounds.

Proposed course of project: Extension of these studies along the lines outlined above are projected and will be executed as appropriate personnel becomes available.

Part C. Honors, Awards and Publications: No entries.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-12-809(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$24,380	$9,740	$34,120	1/3	2	2 1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/2	3 1/3	3 5/6	0

13. BUDGET ACTIVITY:

RESEARCH	/x/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
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Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 852
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Pathologic Anatomy Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Cytodiagnosis Service
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Exfoliative Cytology applied to Human Diagnostic Problems and Research Problems
PROJECT TITLE

7. Dr. Albert W. Hilberg
PRINCIPAL INVESTIGATOR

8. Dr. Roger O'Gara and Dr. Richard Malmgren
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

Not to my knowledge.

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives:

The first and most important objective of the Cytodiagnosis Section is to provide to the clinical staff of the Clinical Center a complete and satisfactory exfoliative cytology service. The second objective is to provide a research tool to be used in various clinical research projects for evaluation of therapy, refinement of diagnosis and assessment of various physiological and anatomical changes in the human body. A third objective is the development and refinement of the techniques and morphological criteria as used in exfoliative cytology so as to further the value and efficiency of the first two stated objectives of the Cytodiagnosis Section.

(Use reverse and additional pages, if necessary)

Methods employed:

The methods involved in this project are primarily those of routine Papanicolaou staining of preparations of human specimens and careful screening and evaluation of morphological changes in cellular material. Of importance to the proper evaluation of these morphological changes are additional methods of special preparation of human materials and special staining techniques applied to the preparations. For example: preparations of some fluid specimens may involve the use of refrigerated centrifugation, differential separation of the strata of sediment obtained by centrifugation, fixation of the material so obtained in various special fixatives (i.e. modified Carnoy, sodium chloride solution, etc.), and then staining the preparations by special staining techniques. Examples of the staining techniques are: Periodic acid Schiff reaction, Kurnick's method for DNA and RNA, Gomori's acid and alkaline phosphatase method, Laidlaw's Method for Dopa oxidase and numerous others.

Patient Material:

The figures compiled below are for only the first ten (10) months of the present calendar year - 1956.

Type of material examined	Accessions	#slides examined	#slides prepared
Vaginal and cervical	1265	2781	2781
Miscellaneous fluids	269	2312	3657
Sputums and Bronchial Washings	144	501	501
Gastric Washings	8	94	94
Prostatic	7	35	35
Total	1693	5723	7068

Institute	Accessions*	Slides*
NCI	1326	4915
NHI	75	308
NIMH	13	49
NIAMD	69	228
NIAID	107	400
NINDB	11	66
NIDR	52	54
No Institute identification	20	70

*Figures for first ten months of 1956 only.

Major Findings:

In respect to diagnostic service to the clinical staff the major findings would concern useful information leading to a diagnosis or to a greater ability to handle therapeutic measures. This is difficult to assess and evaluate in an exact manner. The Cytodiagnosis Service lead to the detection of early diagnosis of several early carcinomas of the cervix, some early tumors of the lung and several sites of metastatic tumor which had been previously undiagnosed. In addition exfoliative cytology provided the first, and in some instances, the only tissue confirmation of neoplastic

disease before death of the patient.

In the wound washing project carried on with the Surgical Branch many operative sites were found to have fragments of tumor tissue in them as determined from the washing of these sites.

The evaluation of the sensitivity response of tissues in the oral cavity to irradiation will, it is hoped, provide a more accurate method of treating patients with oral carcinomas. This work is yet too incomplete to properly evaluate the findings.

Evaluation of estrogenic activity as assessed by morphological changes in cells from the vaginal tract and by special staining of vaginal and oral mucus has provided information to clinicians in cases of amenorrhea, precocious puberty, endocrine therapy, post-menopausal patients, and unusual problems dealing with cyclic changes in endocrine studies.

In regard to the problems of refinement of techniques as used in exfoliative cytology the development of the modified Carnoy fixation technique for the removal of blood and cellular debris from fluid specimens has been a definite step forward in the accurate evaluation of human exudates and washing from various sites. The adaptation of special staining techniques for melanin and for nucleic acids has also provided a useful tool for the advancement of morphological studies of cells.

Significance of the program:

The significance of the diagnostic value of exfoliative cytology to the clinical evaluation of cancer and the early detection of cancer is clearly obvious not only from our own activities in the Clinical Center but from the numerous activities throughout the country.

The significance of the wound washing studies now under way is at least twofold. These studies may provide a prognostic device for the surgeon, but more important they have directed attention to an important area for therapeutic investigation.

The studies involving the sensitivity response to radiation therapy though still in the formative stages could provide an accurate method of determination of the efficacy of this means of therapy in cases of oral cancer. The further evaluation of radiation response after therapy is instituted may also provide information regarding the effectiveness of the therapy. These studies are still too early to make positive statements.

The endocrine evaluation studies involving not only non-malignant cases but also the cases of malignancy treated with hormones provide valuable information to clinicians in the determination of diagnostic problems and for the sequential studies of effectiveness of therapy. The further addition of the study of sex chromosomes, which is now under way on a small scale, enhances the value of the endocrine studies to the clinical staff.

The development of new techniques of preparation and staining of cytologic specimens provides valuable new methods for use in all the studies under way and provides information to other investigators in this field to increase the efficiency and value of exfoliative cytology.

Proposed course of project:

Continuation of complete and adequate service to all the clinical staff of all the categorical institutes in the Clinical Center, National Institutes of Health is our first function. Continued collaboration with the clinical staff in various research projects and the continued search for and development of new preparation and staining techniques is a second and most valuable function for the coming years.

A more intensive investigation of the adaptation of evaluation of sensitivity response of radiation in cases of oral cancer is planned along with evaluation of the radiation response after therapy is given. This project is being carried out with the cooperation of the Radiation Therapy Section.

A continued investigation of the problems involved in wound washings following cancer surgery, with the cooperation of the Surgery Branch, and an amplification of this project to include studies of the vascular drainage of the wound site is also planned.

The evaluation of estrogen activity will continue in all types of endocrine problems and for those cases of endocrine therapy for cancer in which the clinical staff desires such studies. In addition the determination of sex chromosome content of cellular material will also continue.

A very important phase of the work of this laboratory is the continued refinement of techniques of preparation and staining of cytologic specimens and the development of new techniques in order to further clarify and identify the important morphologic changes in cells as a result of neoplastic change or in the instances of modification by hormonal influences.

Evaluation of cellular alterations in gastric mucosa in dystrophic diseases of the nervous system, in cooperation with NINDB, has been started this year and will continue. Reports have indicated the possible significance of these gastric changes in some disease processes.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 852
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$41,840	$16,630	$58,470	1	5	6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	5	6	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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Calendar Year 1956

PUBLIC HEALTH SERVICE--NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 852
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Wound Seeding as a Cause of Operative Failure, A.W. Hilberg, Robert Smith, Ross Miller, Richard Eck, Arthur Ship et al.
(In press) Third National Cancer Congress Proceedings.

2. A New, Inexpensive Circling Device for Marking Specific Areas on Tissue Smears or Sections, P.R. Del Vecchio, E.E. Siegler and A.W. Hilberg
Journal of the National Cancer Institute, February 1956.

3. Cancer-cell Seeding of Operative Wounds, R.R. Smith and A.W. Hilberg
Yearbook of Cancer (In press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

1. Gave talk on "Wound Seeding as a Cause of Wound Failure" at the Third National Cancer Congress, Detroit, Michigan, May 1956.

2. Article "Cancer-cell Seeding of Operative Wounds" Smith and Hilberg, published in the Journal of the National Cancer Institute was selected by the committee compiling the Yearbook of Cancer as one of the outstanding articles for publication in the yearbook.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI 853
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Pathologic Anatomy Branch
LABORATORY, BRANCH, OR DEPARTMENT

4. Surgical Pathology & Postmortem Service
SECTION OR SERVICE

5. **LOCATION (IF OTHER THAN BETHESDA)**

6. No specific research projects in this Branch. However, the diagnostic service is designed to aid the investigative efforts of the N.I.H. staff responsible for patient care. In many instances, investigative study of pathological tissues is of great importance to clinical research.
PROJECT TITLE

7. See Page 1-A
PRINCIPAL INVESTIGATOR

8. ---
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

No specific research projects. The services and diagnostic functions of the Department during 1956 have included:

a. 192 autopsy examinations (from January 1, 1956 through November 30,1956)
Since July 1, 1956 when the first resident class was started in the Department of Pathologic Anatomy, the three residents have performed approximately three-fourths of the autopsy examinations. The rest of the staff have performed a variable number of autopsy examinations. The supervision of the autopsy service is divided between Doctors Thomas and Edgcomb.

(Use reverse and additional pages, if necessary)

7. PRINCIPAL INVESTIGATOR

Dr. Harold L. Stewart, Chief, Pathologic Anatomy Branch
* Dr. Louis B. Thomas, Head, Surgical Pathology & Post-Mortem Service
* Dr. Albert W. Hilberg, Head, Cytodiagnosis Service
* Dr. John H. Edgcomb, Pathologic Anatomy Branch
* Dr. Frederick A. Fox, Resident, Pathologic Anatomy Branch
* Dr. Philip H. Geisler, Resident, Pathologic Anatomy Branch
* Dr. Hugo C. Pribor, Resident, Pathologic Anatomy Branch
\+ Dr. Alan S. Rabson, Laboratory of Pathology, N.C.I.
\+ Dr. Richard L. Swarm, Laboratory of Pathology, N.C.I.
\+ Dr. Clyde J. Dawe, Laboratory of Pathology, N.C.I.
\+ Dr. Eli M. Nadel
\+ Dr. William G. Banfield, Laboratory of Pathology, N.C.I.
\+ Dr. Leon Sokoloff, Laboratory of Pathology & Histochemistry, N.I.A.M.D.
\+ Dr. Harold R. Stanley, Oral Pathologist, N.I.D.R.
\+ Dr. Igor Klatzo, Head, Clinical Neuropathology Section, N.I.N.D.B.

* -These pathologists spend full time in the service and diagnostic functions of the Pathologic Anatomy Department, Clinical Center (Pathologic Anatomy Branch, N.C.I.)

\+ -These associate pathologists spend only part time in the activities of the Pathologic Anatomy Department, principally in the performance of postmortem examinations.

b. 1789 surgical pathology accessions (January 1 through November 30, 1956)
Most of these specimens are now initially described by the resident staff and then their reports are checked by Doctors Thomas and Edgcomb. Doctors Sokoloff, Swarm, Rabson and Stanley also participate in this service.

The objectives of the Surgical Pathology and Post-Mortem Service in the Clinical Center are twofold: first, to furnish a diagnostic service in autopsy and surgically removed tissues, and second, to aid from a morphological standpoint the various clinical research problems which are under study in the Clinical Center.

The methods used are those standard methods which are used in the description of organs and tissues, the fixing and sectioning of this material and the preparation of histological slides. A wide variety of special staining procedures are used in the histopathology laboratory of the Department. The appended chart shows the cumulative total of work performed in the Surgical Pathology and Post-Mortem Services of the Department for 11 months of 1956.

In addition to the diagnostic services, there are several other functions of the Department which have continued and expanded during the past year. At present there are thirty-five scientists in the various laboratories at N.I.H. who have requested particular types of tissue from surgical and postmortem specimens examined in this Department. On many occasions it is possible to furnish these investigators with fresh human material.

The staff of the Department continues to take active part at numerous Clinical Center staff meetings, and in addition, routinely conducts four Departmental meetings weekly. These are the Brain Cutting Conference on Monday, the Autopsy Conference on Tuesday, the Joint Pathology Staff Conference on Wednesday and the Surgical Pathology Conference on Friday.

NCI 853

REPORT OF PATHOLOGIC ANATOMY DEPARTMENT

Annual 1956

INSTITUTE AND TYPE OF PATIENT

Service & Type of Activity	NCI		NHI		NIAMD		NIAID		NIMH		NINDB		NIDR		TOTAL		REF	
	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU	IN	FU
Postmortem-Pathologic Anatomy Service																		
# of autopsies performed	133		29		8		8				10		1		192		3	
Blocks of tissue	8015		1651		471		503				527		37		11227		23	
Slides	18645		4225		1256		1344				1441		109		27097		77	
Slides actually stained	10941		2231		711		960				900		94		15867		30	
Surgical Pathology																		
Tissue Specimens	973		139		112		180		9		47		94		1789		235	
Slides H-E staining	4566		544		633		490		43		159		205		7943		1303	
Frozen Sections	122		13		9		6				4				154			
Special Procedures	817		205		255		321		4		72		20		1786		92	

223 cell blocks, 1416 slides; 52 OPC biopsies, 52 H & E's prepared for Cytodiagnosis Section.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI 853
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$118,540	$47,190	$165,730	5	12	17

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	5	12	17	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

None.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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Calendar Year 1956

PUBLIC HEALTH SERVICE--NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI 853
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Progestational Effectiveness of 19-nor-Ethinyl Testosterone by Oral Route in Women, Hertz, Roy; Waite, John H. and Thomas, Louis B., Proc.Soc.Exp.Biol.Med. 91:418: 1956.

2. Clinico-Pathologic Conference; A case of Bacterial Aortitis Following Resection of Coarctation of Aorta, Terry, Luther; Haverback, B.J. and Thomas, L.B., to be published in Heart Bulletin.

3. Mycotic Endocarditis, A Review and Report of A Case Due to Cryptococcus Neoformans, Lombardo, T.A.; Rabson, Alan S. and Dodge, Harold T., Am. J. Medicine (In press).

4. Studies on the Use of Viruses in the Treatment of Carcinoma of the Cervix, Smith, Robert R., Huebner, Robert J., Rowe, Wallace P., Schatten, William E. and Thomas, Louis B., Cancer, November-December, 1956.

5. Some Aspects of the Pathology of Collagen Diseases, Sokoloff, Leon, Bull. N.Y. Acad. Med. 32: 760-767, 1956.

6. Biopsy as a Diagnostic Procedure in Rheumatic Diseases, Sokoloff, Leon, Bull.Rheum.Dis. 7, Supplement to Oct.1956.

7. The Pathology of Gout, Sokoloff, Leon, Metabolism (In press).

8. Vascular Lesions in Rheumatoid Arthritis, Sokoloff, Leon and Bunim, Joseph J., J.of Chr.Dis. (In press).

9. Systemic Lupus Erythematosus, Bunim, Joseph J., Harvey, A. McGhee, Bollet, Alfred J., Hilbish, T.F., Van Scott, Eugene, Sokoloff, Leon and Brecher, George, Circulation 14, 125-134, 1956.

10. The Value of Step Serial Sectioning in the Morphologic Study of Human Periodontal Disease, Stanley, Harold R., Jr., J. of Periodontology (In press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

1. Lecture entitled, Aspiration Biopsy, as a part of the Course in Surgical Pathology sponsored by the International Academy of Pathology, Cincinnati, Ohio, April 1956 by Louis B. Thomas.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-900
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Physiology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Growth Stimulating Factor(s) present in Walker Carcinoma 256
PROJECT TITLE

7. Julius White and F. K. Millar
PRINCIPAL INVESTIGATOR

8. G. B. Mider and Robin Brooks
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None.

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To continue the studies of the growth-stimulating factor present in Walker carcinoma 256.

Methods Employed: Fractionation of the Walker 256 with the purpose of isolating the growth factor.

Major Findings: Animals bearing the transplanted Walker tumor grow larger tumors with no added loss in body weight when ingesting a 24% tumor diet as compared with similar animals ingesting a 20% casein diet.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-900
SERIAL NUMBER

Significance to the program of the Institute: There is present in tumor tissue a factor(s) which stimulates growth when fed, spares nitrogen and apparently has a high food efficiency value.

Proposed course of project:

1. To make an intensive fractionation of the active principles and possible identification.

2. To extend this study to other tumors.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-900
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$52,200	$20,800	$73,000	1-2/3	3	4-2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1 2/3	3	4 2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSIST-ANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

(Use reverse and additional pages, if necessary)

Form No. CRP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-901
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Physiology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Probable Sources of Available Nitrogen for Tumor Growth
PROJECT TITLE

7. Julius White
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine the availability of nitrogen for the rapidly growing transplanted Walker carcinoma 256.

Methods Employed: Young, growing, as well as adult, rats were placed on a 20% casein diet of known composition. The Walker carcinoma 256 was implanted subcutaneously and, at the same time, N^{15} glycine was incorporated into the diet. At various intervals the animals were sacrificed and the total nitrogen balance was determined.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-901
SERIAL NUMBER

Major Findings:

1. Confirmed earlier observations that the tumor and host store nitrogen at approximately the same rate in small tumors (8 grams).

2. No change in uptake in host and tumor is noted in animals in which the tumor is 10 percent of the weight of the host.

3. Preliminary observations seem to indicate that there is a more rapid uptake of N^{15} by the tumor than the host when the tumor is 25 percent of the weight of the host. These observations are yet to be confirmed.

Significance to the program of the Institute: To obtain a better understanding of the tumor-host relationship. At what stage of tumor growth does the metabolic insult appear in the host? Is the nitrogen present in tumor tissue available under some circumstances for growth of the host? Are the "building blocks" used by the tumor similar to those used by the host?

Proposed course of project: To determine the utilization of available N^{15} in animals whose energy demands exceed its intake and to determine where and how this imposition on the host occurs.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-901
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$33,560	$13,370	$46,930	2/3	2-1/3	3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2/3	2 1/3	3	0

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-902
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Physiology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Alterations in Nucleic Acid and Protein Syntheses in the Rat Small Bowel Epithelium Following X-Irradiation.
PROJECT TITLE

7. Jane N. Toal and Julius White
PRINCIPAL INVESTIGATOR

8. R. Bland Williams and H. Carpenter, N.N.M.C., Bethesda, Md. Naval Medical Research Institute,
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None.

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: Nucleic acid and protein syntheses in the rat small bowel epithelium following x-irradiation are being studied in conjunction with Dr. R. Bland Williams' morphologic studies on the role of prolongation of interphase in the recovery of this tissue after threshold to tissue lethal doses of x-ray.

Methods Employed: Rats were sacrificed after several time intervals for each dose of x-ray. The RNA was examined as individual nucleotides (i.e. cytidylic acid, adenosine 5 phosphate, adenylic acid, uridylic acid, and guanylic acid). For the DNA only the purines (i.e. adenine and guanine) were examined. Since the rats had received glycine N^{15} intraperitoneally five hours before sacrifice,

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd): NCI-902
SERIAL NUMBER

the concentration of N^{15} found in the nucleotides and purines was illustrative of the amount of synthesis taking place during the last 5 hours. The behaviour of protein, PNA, and DNA following x-irradiation is expressed as the per cent of the N^{15} concentration of an unirradiated control.

The small bowel tissue was defatted and the nucleic acids and protein were prepared by a modified Schmidt-Thannhauser procedure. The PNA nucleotides were separated and purified by chromatography on Dowex 2 resin, the DNA purines on Dowex 50 resin. Kjeldahl nitrogen and atom per cent excess N^{15} determinations were done on all fractions.

Major Findings: This project is nearly complete and the findings are summarized here.

Following x-irradiation there is an initial dose dependent depression in DNA synthesis due to the acute cell death of some antemitotic cells. However, a considerable amount of synthesis (as much as 90 per cent after 150 r) is still going on. The DNA synthesis continues to fall in spite of the fact that some cells have resumed division in an abortive recovery. Synthesis remains at a low plateau during the second period of mitotic suppression and begins to recover shortly before the recovery of cell division can be seen. During this final sustained recovery, DNA synthesis rapidly increases to as much as 600 per cent above control levels and more slowly regains normal levels.

The protein and PNA from the irradiated animals remain similar to control values with the exception of a moderate increase during the final recovery at which time the mitotic index and DNA are at their highest levels.

Significance to the program of the Institute: When this chemical study is presented with the morphologic study, it will be the first detailed correlation of changes in cell division with alterations in nucleic acid metabolism in a mammalian tissue following irradiation.

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10. PROJECT DESCRIPTION (Cont'd):

NCI-902
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These studies were undertaken to obtain a better understanding of the mechanisms by which x-radiation interferes with cellular proliferation. When ionizing radiation is used in the treatment of certain types of cancer such knowledge of the tissues involved is necessary to select the most effective dose and time sequence.

Proposed course of project: These methods will be continued to study the effect of fractionating an x-ray dose, the effect of radio mimetic chemicals, and the effect of x-ray on the precursors of DNA.

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INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-902
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$18,640	$7,420	$26,060	2/3	1	1-2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2/3	1	1 2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSIST-ANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

(Use reverse and additional pages, if necessary)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-904
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5. Department of Biology, Princeton University, Princeton, N. J.
LOCATION (IF OTHER THAN BETHESDA)

6. Study of Biological Effects of Ultraviolet Irradiation.
PROJECT TITLE

7. Harold F. Blum
PRINCIPAL INVESTIGATOR

8. Prof. E. G. Butler, Mrs. S. E. Schmidt, V. Gregg, J. R. Daube and G. Soffen of Princeton University. Senior students A. A. Bechtoldt, T. B. Dailey and W. G. Ridgeway are also participating in the experimental program.
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None.

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To study the biological effects of ultraviolet irradiation with the ultimate aim of better understanding of the carcinogenic action of that agent. These studies aim to combine an experimental and theoretical approach to the problem of carcinogenesis.

Methods Employed: The approach is a combined theoretical and experimental one. Using data available from our earlier work on the induction of cancer by ultraviolet light, a mathematical model has been constructed which explains certain aspects. Realizing that any such model can only have meaning if related to real biological phenomena, experimental studies of various effects of ultraviolet radiation on living systems have been undertaken.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd):

Our model would indicate that the mechanism of cell proliferation and its control are of particular importance in this problem, and hence the effect of ultraviolet radiation on this phenomenon has been a principal preoccupation. Our studies have extended into related fields as this has seemed indicated.

Major Findings:

I. Studies of regeneration after ultraviolet radiation in the larvae of the urodele amphibian. (In collaboration with Professor E. G. Butler, with the assistance of S. E. Schmidt and V. Gregg.)

The studies described in the last annual report have been incorporated into a paper entitled, "Studies on regression and regeneration in the urodele forelimb after localized ultraviolet radiation" by H. F. Blum, E. G. Butler, J. J. Chang, R. C. Mawe, and S. E. Schmidt, which will be published in the near future in the Journal of Cellular and Comparative Physiology. In a continuation of these studies we have shown that ultraviolet irradiation of the upper arms of salamander larvae has no manifest effect on the regeneration of the lower arm after amputation through unirradiated tissue, indicating the strictly local character of the effects of the radiation. These experiments seem to present conclusive evidence that there is no appreciable spread of effects of ultraviolet radiation in these limbs, by means of circulatory or nervous channels; they have been described in a paper entitled, "The localized character of the ultraviolet effects on the urodele forelimb" by E. G. Butler, H. F. Blum, and S. E. Schmidt, submitted for publication to the Journal of Cellular and Comparative Physiology. Having shown in earlier studies that supernumerary limbs, displaying a variety of abnormalities could be induced by ultraviolet radiation of the elbow region (Butler and Blum, J. Nat. Cancer Inst., (1955) 15, 877-888) we undertook to study the regeneration of these supernumerary limbs after amputation. In studies described in last years report it was shown that when ultraviolet irradiated forearms were amputated the regenerates often displayed abnormalities (when photorecovery was eliminated by keeping the animals in the dark), and parallel to the above investigation we have studied regeneration after a second amputation. In both the case of the supernumerary limbs, and the abnormal regenerates in normal position it was found that blastema formation and regeneration follow amputation, but that there is no tendency to copy the morphological pattern of the abnormality seen in the limb that was amputated. There appears to be some recovery toward the normal condition of the limb. These

10. PROJECT DESCRIPTION (Cont'd):

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investigations are being continued. Systematic histological studies of the irradiated limbs are under way, having particular regard to early changes and to photorecovery.

II. Effects of ultraviolet radiation on mouse skin. (Continued with the collaboration of J. Daube, G. Soffen, T. H. Dailey and V. Gregg.)

A good deal of progress has been made in a descriptive study of the events that follow single doses of ultraviolet radiation of the ear of the albino mouse. Last year an adequate means of obtaining uniform, controlled irradiation was worked out. This year a satisfactory method of histological study of this refractory material has been developed; and a part of the picture worked out. It is hoped that once the immediate descriptive study is completed, including a study of photorecovery, it will be possible to undertake cytochemical and other studies having direct bearing on our previous work on the induction of cancer by ultraviolet radiation. The attempt to work out a satisfactory mathematical description of carcinogenesis by ultraviolet radiation is continuing, with some apparent success; and it seems probable that the present studies may contribute important information in this regard.

III. Studies of hemolysis by ultraviolet radiation and by photosensitization to visible light. (Continued with the collaboration of J. S. Cook, J. Daube, A. A. Bechtoldt, and W. G. Ridgeway.)

In 1954, before leaving for Berne to study with Professor A. Von Muralt, under a U. S. Public Health Research Fellowship, Dr. Cook and I undertook to investigate O_2 dependence of ultraviolet hemolysis. Last year I continued this study with the assistance of Mr. J. Daube. In March of this year Dr. Cook returned to my laboratory under a continuation of the same Fellowship, remaining here into August. Dr. Cook continued the studies we had commenced, including latterly the examination of photosensitized hemolysis. These studies indicate certain amount of O_2 dependence in the ultraviolet spectrum, which varies with wavelength, but is in no case completely O_2 dependent as is hemolysis photosensitized by exogenous sensitizers. The results of these studies are now being prepared for publication. A continuation of studies of photosensitized hemolysis is being undertaken by A. A. Bechtoldt and W. G. Ridgeway under my supervision.

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10. PROJECT DESCRIPTION (Cont'd): NCI-904
SERIAL NUMBER

IV. Work at the Marine Biological Laboratory, Woods Hole.

In collaboration with Mr. Gerald Soffen studies were undertaken of the action on the fertilized Arbacia egg of a substance, Agmatine sulfate, which has been credited with the ability to accelerate cell division. These studies, which resulted from a prediction made by Dr. Norman Anderson of the Oak Ridge National Laboratories (who kindly provided the material) were disappointingly negative. Since Dr. Anderson is presumably continuing studies of the effect of this substance on other material, this finding ought to be kept confidential. Professor Hans Trampusch of the University of Amsterdam spent the summer at the Marine Biological Laboratory as the guest of Professor Butler, carrying out studies of the regeneration of amphibian limbs after x-ray. Such regeneration does not take place ordinarily, but Professor Trampusch has been able to obtain it by transplanting normal amphibian into the irradiated limb.

I had the good fortune in sharing the laboratory with Dr. Trampusch and Dr. Butler to observe at first hand these fascinating experiments, which have not yet been described in detail in print. In order to make room for Professor Trampusch my own research had to be curtailed, but I found the observation of this work at close hand and discussions with Professor Trampusch more than compensated. I devoted a good deal of time to collaboration with Dr. Butler in reviewing our joint research, and preparing the material for publication, to reading and to analysis of my own data.

Significance to the program of the Institute: It is our conviction that these studies of the action of a specific readily controllable carcinogenic agent, including the various biological effects of that agent, must ultimately give us much information concerning the mechanism of carcinogenesis in addition to what they have already contributed.

Proposed course of project: Further experiments are under way dealing with the effects of ultraviolet radiation on regeneration. Increased emphasis is being placed on studies of the effects of ultraviolet radiation on mouse skin, with particular interest in the hyperplasia which follows exposure to that agent, and its subsequent controlled return toward normal. Mr. Gerald Soffen, who has had previous

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10. PROJECT DESCRIPTION (Cont'd):

NCI-904
SERIAL NUMBER

experience in the study of effects of ultraviolet radiation on skin, has come to Princeton to work under my supervision for the Ph.D. Degree, electing this subject for his thesis study. His addition to this project should greatly assist our efforts.

Studies of photosensitized hemolysis are being continued by students under my supervision. These should further our knowledge of radiation-induced cytolysis.

The synthetic approach to the problem of carcinogenesis by ultraviolet radiation will continue.

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INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-904
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,190	$4,440	$15,630	1	-	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	0	1	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NCI-904
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Blum, H. F.: The photochemical basis of photopathology. Proc. 1st International Photobiological Congress, Amsterdam 1954; pp. 359-368. (Invited paper.)

Cook, J. S.: Some characteristics of hemolysis by ultraviolet light. J. Cell. and Comp. Physiol. 47: 55-84, 1956. (Paper describing work carried out under my supervision.)

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Honors and Awards: Elected Trustee of the Marine Biological Laboratory, Woods Hole.

Lectures and Symposia:

1. Lecture entitled "Beyond Natural Selection," Bryn Mawr College, under the auspices of the Sigma Xi Chapter on January 17, 1956.

2. Paper entitled "On the Origin of Replicating Systems," Annual Growth Symposium of the Growth Society at Providence, R. I., on July 19-20, 1956 (by invitation).

3. Physics Seminar, Princeton University, on November 15, 1956.

4. Lecture entitled "The Transition from Non-Living to Living," honoring the Geophysical Year organized by the Sigma Xi Chapter, University of Kansas, on December 1, 1956.

5. Address entitled "Skin Color and Natural Selection," Evolution Society of the University of Kansas on December 2, 1956.

6. Chairman of Symposium entitled "Modern Ideas of Spontaneous Generation," organized by the New York Academy in collaboration with the American Association for the Advancement of Science on December 26, 1956. (By invitation.)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-905
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Physiology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Iodide Concentrating Mechanism of the Thyroid Gland
PROJECT TITLE

7. S. H. Wollman
PRINCIPAL INVESTIGATOR

8. J. C. Reid
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

This project complements research done in the Clinical Endocrinology Branch of N.I.A.M.D. - Project No. A2201

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To learn about a) the mechanism by which the thyroid gland maintains a concentration of radioiodide elevated above that in the blood, and b) the way in which the radioiodide and stable iodide concentrations in the thyroid influence the uptake of radioiodine.

Methods Employed: Kinetic studies are made of the concentration of radioiodide in the thyroid, the rate of binding of radioiodine, and the influence of inhibitors and various physiological states of the animal on these processes.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd): NCI-905
SERIAL NUMBER

Major Findings: A study was made of the rate of binding of radioiodine. Binding rates were expressed as the ratio of the amount of radioiodine bound per minute to the amount of radioiodide present in the thyroid (binding rate constant). Binding rate constants were 0.5 and 1.5 respectively in mice and rats fed moderately low iodine diets. The binding rate constant was markedly reduced in hypophysectomized mice but only moderately in hypophysectomized rats. After hypophysectomy the thyroid radioiodide concentration rose in mice but fell in rats.

The effect of thiocyanate on the binding rate constant was studied. The results may be interpreted as indicating that the actual binding mechanism was not inhibited by thiocyanate, but that transport of iodide to the binding site was inhibited.

It was also found that radioactive thiocyanate was not concentrated by the thyroid gland of mice. In conjunction with earlier observations the present results appear to eliminate simple adsorption as the mechanism by which radioiodide is concentrated by the thyroid.

Significance to the program of the Institute: This work is leading to an understanding of the relative importance of various factors in controlling the accumulation of radioisotopes by tissues.

Proposed course of project: This project will be continued with somewhat more emphasis on investigation of the role of blood flow to the thyroid in the accumulation of radioiodine by the thyroid gland.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-905
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$18,640	$7,420	$26,060	2/3	1	1-2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2/3	1	1 2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI-905
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Wollman, S. H.: Nature of the inhibition by thiocyanate of the iodide concentrating mechanism of the thyroid gland. Am. J. Physiol. 186: 456-459, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-906
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Physiology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Radioiodine Metabolism of Transplantable Thyroid Tumors
PROJECT TITLE

7. S. H. Wollman
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None.

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: To compare iodine metabolism in thyroid tumors and normal thyroid.

Methods Employed: Iodine metabolism was studied using radioactive iodine.

Major Findings: A number of different lines of transplantable thyroid tumors in the Fischer rat have been tested for functional activity using radioiodine. Tumors exist with a wide range of activities. Some are more active than the thyroid in the same host, even when allowance is made for differences in weight. Some of the independent tumors can concentrate radioiodide. In both of these latter

(Use reverse and additional pages, if necessary)

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10. PROJECT DESCRIPTION (Cont'd): NCI-906
SERIAL NUMBER

properties the rat tumors seem to differ significantly from mouse thyroid tumors.

Significance to the program of the Institute: These studies indicating strain differences in thyroid tumors force us to recognize the need for caution in generalizing to man from results obtained on a single animal species.

Proposed course of project: Studies of the radioiodine metabolism of thyroid tumors in the rat will be continued.

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INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-906
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,190	$4,440	$15,630	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 and 17.

(Use reverse and additional pages, if necessary)

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-909
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Physiology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Metabolic Fate of the Histidine Side Chain.
PROJECT TITLE

7. James C. Reid
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The purpose of this project is to acquire more information on the metabolic fate of the side chain of histidine. Certain aspects, i.e. interrelationships with other amino acids have already been reported. Subsequent work has concentrated on testing a hypothesis that the side chain might be a ribose precursor. This aspect was actually the major one in the genesis of the project, but ambiguities in the experimental results hitherto prevented the reaching of definite conclusions.

Methods Employed: The problem is being studied with the aid of radioactive carbon and tracer methods.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd): NCI-909
SERIAL NUMBER

Major Findings: Certain difficulties which developed in the optical resolution of labeled DL-histidine were worked out and a quantity of the pure L-form was prepared.

The L-histidine-alpha-C^{14} was used to carry out a definitive experiment to check earlier observations. As observed before with DL-histidine, it was found that nucleotide fractions derived from liver ribonucleic acid of rats given labeled L-histidine are strongly radioactive when isolated on Dowex-1. Purity checks, however, definitely show that most of the radioactivity is not in the nucleotides themselves.

The alpha carbon of histidine is therefore not a precursor of ribose (or of nucleotide bases). At the same time, the pattern of other amino acids in which isotope is observed to accumulate indicates that this carbon does enter the Krebs cycle. There is little difference apparent between the behavior of L- and DL-histidine, which may mean that the deamination step occurs very early in the metabolic sequence.

Significance to the program of the Institute: This project is of the academic type, designed to increase general knowledge of the cancer problem. The findings relate to protein and nucleic acid metabolism, both important topics in the biochemistry of cancer.

Proposed course of project: The detection of major unidentified metabolites of histidine has come out of the project as a by-product. Their chromatographic behavior and other properties suggest that they may be of a type different from any which might be expected on the basis of existing knowledge of histidine metabolism, and suggest also that they may be identical with unidentified materials observed by others to occur in Schmidt-Thannhauser tissue extracts. During the coming year it is proposed to collect more information on these substances in an attempt to identify them.

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INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-909
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,180	$4,440	$15,620	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(Use reverse and additional pages, if necessary)

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INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI-909
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Reid, James C., Temmer, Odette S., and Bacon, Martha O.: The metabolism of L-Histidine-2-C^{14} in the normal and hepatoma-bearing rat. II. Protein and nucleic acid relationships. J. Nat. Cancer Inst. 17: 189-201, 1956.

(2) Reid, James C.: Radioactivity and tracer studies. The Science Teacher 23: 137-139, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Reviewed by James C. Reid: "Radioisotopes in Biology and Agriculture," by C. L. Comar. Capital Chemist 6: 105, 1956.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-910
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Physiology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Tumor Autoradiography.
PROJECT TITLE

7. James C. Reid
PRINCIPAL INVESTIGATOR

8. Julius White, G. B. Mider
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: It is desired to obtain information on the circulation pattern in tumors and on the stability of tumor protein.

Methods Employed: Animals bearing tumors are dosed with radioactive amino acids and after various periods of time the tumors are removed and sliced. The slices are fixed and placed in contact with x-ray film, thereby registering an image of the distribution of isotope across the slice.

(Use reverse and additional pages, if necessary)

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10. PROJECT DESCRIPTION (Cont'd): NCI-910
SERIAL NUMBER

Major Findings: Experiments made with glycine, previously reported, have been repeated with uniformly labeled L-lysine in the Walker tumor. The results are qualitatively the same as before and it is therefore indicated that they represent the behavior of amino acids in general rather than being unique to glycine. Quantitative differences which appear to exist are probably within the range which can be accounted for by differing degrees of metabolic combustion of the two amino acids and by differences of the content of them in the tumor proteins.

Interpretation of experiments lasting for comparatively long time intervals has been beclouded by the excessively necrotic character of the Walker tumor. Accordingly, a search has been made for tumors with minimal necrosis. Three have been selected: the ACMCA II fibrosarcoma in the Irish rat; the S91 melanoma in the DBA hybrid mouse; and the mammary adenocarcinoma C3HBA in the C3H/An mouse. A repetition with these tumors of the experiments done so far with the Walker tumor is in progress.

Significance to the program of the Institute: The question of the relationship between the nutrition of a tumor and that of its host is an important one in understanding the uncontrolled growth of tumors. These experiments represent one approach to this question. The information gained is also of interest in connection with the question of how necrosis affects the overall metabolism of a tumor. This problem is a troublesome one in the investigation of tumor chemistry.

Proposed course of project: It is expected that the data obtained from the experiments now in progress will establish the observations made so far on a sufficiently secure basis to proceed to "saturation" experiments in which administration of isotope is in multiple rather than single doses.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-910
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	0	0	0	-	-	-

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	0	0	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-911A
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Energy Metabolism Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Energy Metabolism--Instrument Calibration and Standardization.
PROJECT TITLE

7. A. W. Pratt, W. C. White and B. E. Burr
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None.

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: The over-all objective of this program constitutes the continuous, accurate measure of the energy metabolism of small experimental animals under different experimental conditions for prolonged experimental periods.

The immediate objective of this reporting period relates to the accuracy of the total procedures and, specifically, the application of the analytical respirometers in the continuous routine study of tumor-bearing animals.

(Use reverse and additional pages, if necessary)

10. PROJECT DESCRIPTION (Cont'd):

Major Findings:

(1) Instrumentation, Calibration and Standardization.

At the end of the previous reporting period it was stated that the analytical respirometers developed by our group were electrically and mechanically ready for routine use in the study of energy metabolism of rodent experimental animals. Our experience with the instruments during this reporting period has confirmed the expected performance. Calibration and standardization of the instruments assume, therefore, considerable importance for intelligent interpretation of the data obtained from the analytical respirometers. Accordingly, a major effort has been made to define precisely the errors associated with the standardizing and calibrating procedures.

(2) Mass Spectrometer Studies.

Mass spectrometric analysis is the basic measure to which our analytical respirometers are referred. The many advantages inherent in such a system are apparent. The objective has been to achieve accurate and precise measure of all components of the respiratory exchanges of the experimental animals including nitrogen, oxygen, argon, carbon dioxide and any gaseous metabolite which may be excreted via the respiratory exchanges.

Careful experimentation has led to the derivation of new mathematical relations for calculation of total pressure from the micromanometer readings. The correction of this basic measure has eliminated a major error within the calibration and standardization procedures.

Mass spectrometric analysis of outside air regularly gives CO_2 values which are higher than the accepted values. These experimentally-obtained values have been seen to vary as a function of the degree of gas sensitivity of the filament of the mass spectrometer, partial pressure of oxygen, etc. Study has shown that molecular oxygen will react with the tungsten carbide filament with a yield of CO and CO_2. This reaction has been investigated quantitatively since this internal conversion, although variable, has been shown to increase the calculated $\Delta CO_2/\Delta O_2$ ratios by as much as 1%.

10. PROJECT DESCRIPTION (Cont'd)

The precision attainable in routine mass spectrometric analysis of respiratory air has been studied experimentally. The experimental series reveals standard deviations as follows:

Nitrogen	±	.014 Mol %
Oxygen	±	.014 Mol %
Argon	±	.005 Mol %
Carbon Dioxide	±	.007 Mol %

These values thus represent the routine precision which can be achieved in mass spectrometer analysis.

(3) Analytical Respirometer Studies.

Associated with the routine use of the analytical respirometers has been the continued refinement of the physical measurements and the technical procedures. Steps have been taken to monitor accurately the flow rates, variations in barometric pressure, variations in temperature, etc. Additionally, the weighing and handling procedures for diet, urine, feces, and water have been carefully examined. It is difficult to express the possible error contribution of each individual measurement or procedure. Taken together, however, it can be shown that the weight change of the animal agrees within 1% of the weight difference of substance taken in by the animal less the substance excreted by the animal. Also, the body-weight gain of our experimental animals, maintained routinely in the metabolic chamber, is indistinguishable from body-weight curves for companion animals maintained in a well-tended animal room on the same diet.

These data allow the conclusion that the analytical respirometers, together with their ancillary equipment afford a satisfactory experimental system for the study of energy metabolism of rodent experimental animals.

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-911A
SERIAL NUMBER

Significance to the program of the Institute: Study of the effects on the energy metabolism of a host animal, following tumor implant and during subsequent tumor growth, promises to yield significant information bearing on host-tumor relationships. The development of these new techniques for these studies should allow an accumulation of whole animal data which, together with the advances in intermediate metabolism, should increase our understanding of the pathological physiology of the tumor process.

Proposed course of project: Continuing evaluation of accuracy will be necessary in this program.

Calendar Year 1956

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-911A
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$22,380	$8,920	$31,300	1-2/3	1/3	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1 2/3	1/3	2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 and 17.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-911B
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Energy Metabolism Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Energy Metabolism--Animal Findings and Future Instrumentation.
PROJECT TITLE

7. A. W. Pratt
PRINCIPAL INVESTIGATOR

8. W. C. White
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The over-all objective of this program constitutes the continuous accurate measure of the energy metabolism of small experimental animals under different experimental conditions for prolonged experimental periods.

The immediate objective of this reporting period relates to the performance of the total procedures and specifically the application of the analytical respirometers in the continuous routine study of tumor-bearing animals.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd.)

NCI-911B
SERIAL NUMBER

Methods Employed: These studies are being carried out using the analytical respirometers developed in this laboratory in addition to the usual, routine analytical chemical methods. The animals receive a 20% casein diet with vitamins and mineral supplements adequate to give excellent growth. Tumor growth has not been allowed to exceed 15% of total-body weight.

Major Findings: The relatively long-term energy metabolism changes appear to be qualitatively similar for all animals studied. These changes are:

(a) Increased oxygen uptake following tumor transplant and during tumor growth.

(b) Decreased carbon dioxide excretion during tumor growth in proportion to the increase in oxygen consumption with resulting decrease in R.Q.

(c) Increased total energy expenditure during tumor growth.

(d) Increased non-protein energy metabolism contributes most of the increased total energy expenditure.

It has been tentatively concluded that the retention of carbon probably in the net form of carbon dioxide is of primary importance during tumor growth. Reasons for this conclusion are fully discussed in a publication which is now in manuscript.

Present Instrumentation: To better understand the energy metabolism of the tumor-bearing animal it is necessary to achieve accurate measure of heat production directly. To this end, effort is being made to build a direct calorimeter which will be adiabatic and suitable for long-term study of the experimental animals. While many technical problems remain unsolved, sufficient progress has been made to feel that a true correlation between the indirect measure and the direct measure of heat production of the experimental animal can be achieved this coming year. Such a correlation should lead to greater understanding of the animal's bio-energetics in relation to the changes in energy metabolism related to tumor growth.

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd):

NCI-911B
SERIAL NUMBER

Significance to the program of the Institute: Study of the effects on the energy metabolism of a host animal, following tumor implant and during subsequent tumor growth, promises to yield significant information bearing on host-tumor relationships. The development of these new techniques for these studies should allow an accumulation of whole animal data which, together with the advances in intermediate metabolism, should increase our understanding of the pathological physiology of the tumor process.

Proposed course of project: See Present Instrumentation under Major Findings.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-911B
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$26,110	$10,390	$36,500	1	1-1/3	2-1/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	2 1/2	3 1/2	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-911C
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Energy Metabolism Section
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Energy Metabolism--Body Composition Studies.
PROJECT TITLE

7. A. W. Pratt and F. K. Putney
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: Studies of the body composition of experimental animals have been undertaken as part of the energy metabolism project. In particular, the separation of fat and non-fat fractions from lyophilized material and the determination of caloric content and nitrogen percentage provide data which can be correlated with the values calculated from indirect calorimetry measurements for energy retained by the animal during the experimental period.

Methods Employed: Routine chemical methods, bomb calorimetry.

Major Findings: The first series studied, which included both nor-

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-911C
SERIAL NUMBER

mal and tumor-bearing animals, showed that for our standard diet the caloric content of both fat and non-fat fractions of lyophilized carcasses is relatively constant except in the case of animals with very large tumors. Thus, the percent contributed by each fraction may be estimated from the caloric value of the total. The nitrogen concentration in the defatted portion is also constant while that in carcass fat is negligible, so that another estimate of the fat fraction may be made from the nitrogen percent of the total. The weights obtained in the first series and the calculated results were all in good agreement.

After the first series, a different procedure for fat extraction was adopted yielding fats of higher caloric value. The analysis of fat for fatty acids and unsaturation was also undertaken.

Studies are now in progress on several series of animals in order to determine the relation of fat content to caloric values and nitrogen percentage for normal and tumor-bearing animals on casein or tumor diets. This information will be important in the subsequent calculation of the bio-energetics of the experimental animal.

Significance to the program of the Institute: Study of the effects on the energy metabolism of a host animal, following tumor implant and during subsequent tumor growth, promises to yield significant information bearing on host-tumor relationships. The development of these new techniques for these studies should allow an accumulation of whole animal data which, together with the advances in intermediate metabolism, should increase our understanding of the pathological physiology of the tumor process.

Proposed course of project: Immediate plans include the determination of the ash content of the body fractions.

Further developments will lead to a study of carbon balance.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-911C
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$29,840	$11,880	$41,720	1-1/3	1-1/3	2-2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2 1/3	1 1/3	3 2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-914
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Physical Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. In Vivo Measurement of Tumor pH with the Capillary Glass Electrode.
PROJECT TITLE

7. H. Kahler
PRINCIPAL INVESTIGATOR

8. B. Haines
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The object of this project is to determine the in vivo pH of tumors under different experimental conditions.

Methods Employed: Following anesthesia, three fine-tipped capillary electrodes were inserted into each tumor in selected sites, one electrode was inserted into normal leg muscle, and the pH was recorded by automatic equipment over a period of 18 hours. Fine thermocouples were also inserted into selected sites such as skin, tumor, muscles and rectum, and the temperatures were recorded on another instrument throughout the experiment. After an initial period, an injection of one of numerous sugars was made. At the conclusion of the pH run the tumors were grossly

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-914
SERIAL NUMBER

examined for tissue homogeneity for comparison with the pH results. Then they were analyzed for lactic acid by a colorimetric method.

Major Findings: In a given tumor the pH varies from region to region. This appears to be correlated with necrosis of the tissue and position of the region with respect to the blood supply. The lactic acid determinations roughly correlate with the pH measurements.

The temperature measurements indicate that non-infected tumor skin is 2^{o} cooler than normal skin in the same animal.

Significance to the program of the Institute: The pH of a tissue is one of its basic properties and where this pH deviates from the normal tissue value the detailed study of these changes is essential to an understanding of tumor chemical processes.

Proposed course of project: The detailed study of pH versus lactic acid content in different regions of a tumor will be continued and in some cases compared with microscopic findings.

Calendar Year 1956

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-914
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$22,380	$8,910	$31,290	1/3	1-2/3	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2 2/3	3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-915
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Physical Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Physical Characterization of Cellular Bodies Diffusion of DNA
PROJECT TITLE

7. H. Kahler
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To measure the diffusion constant of DNA at different concentrations.

Methods Employed: Solutions of dialyzed DNA were set up in a diffusion cell and an interference method (Rayleigh) used to determine the concentration distribution changes with time. From this the diffusion constant can be determined and its variation with concentration.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd)

NCI-915
SERIAL NUMBER

Major Findings: The extrapolated diffusion constants combined with previously determined sedimentation constants have made possible the calculation of the molecular weight of a modern DNA preparation. This value appears to be in agreement with light scattering values.

Significance to the program of the Institute: The properties of DNA are thought to be of significance to genetics and cancer.

Proposed course of project: A sufficient number of determinations of the diffusion constant will be made so that an estimate of the standard deviation of the results may be obtained. This will require the greater part of the following year to complete.

Calendar Year 1956

Form No. ORP-1
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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-915
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,460	$2,970	$10,430	1/3	1/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-916
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Physical Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Characterization and Comparison of Nucleic Acids and Nucleoproteins of Normal and Neoplastic Tissues.
PROJECT TITLE

7. Joseph Shack
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The long term objectives remain the same as outlined in last year's report on this project. The immediate objective of the work done this year was to develop methods of determining whether a given sample of nucleate was to any extent denatured, and if so, how much.

Methods Employed: Various procedures for isolation of nucleic acid; selective denaturation by exposure of the nucleate to heat, acid, alkali, etc. Characterization of the native and denatured nucleates by viscometric, titrimetric, light absorption and other physico-chemical means.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-916
SERIAL NUMBER

Major Findings: Two new procedures were developed for determining 1) whether a sample of nucleate is to any extent denatured and 2) how much denatured material it contains. These procedures are based on previous findings in this project that the ultraviolet absorption of denatured nucleate varies with 1) temperature and 2) salt concentration under conditions where that of native nucleate does not.

Significance to the program of the Institute: In order to make significant comparisons of nucleates from different sources (e.g. normal and malignant tissues) it is necessary to be certain that neither have undergone denaturation during the isolation procedures.

Proposed course of project: To continue with isolation and characterization of nucleic acids from various and malignant tissues.

Calendar Year 1956

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-916
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,450	$2,970	$10,420	1/3	1/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-917
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Physical Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Characterization and Comparison of the Deoxyribonucleases and Deoxyribonuclease Inhibitors of Malignant and Normal Tissues and Body Fluids.
PROJECT TITLE

7. Joseph Shack
PRINCIPAL INVESTIGATOR

8. Jesse Steinfeld of General Medicine Branch, NCI (in that part of project involving human samples)
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To determine the nature, quantity and properties of the deoxyribonucleases (DNase) and DNase inhibitors of normal and malignant tissues and of body fluids.

Methods Employed: Extracts of tissues and preparations of body fluids suitable for enzyme assay are made. Enzymatic activities determined under various conditions by suitable methods (e.g. viscosity, solubility, light absorption).

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-917
SERIAL NUMBER

Major Findings: Studies of the deoxyribonucleases of human serum and urine have shown that:

a) Serum contains DNase I but no DNase II; a heat labile inhibitor of DNase I is present in fresh sera.

b) Urine contains no DNase II but considerable DNase I. The activity of DNase I in urine may be up to 60-100 times that in serum. Normal human urine does not contain DNase inhibitors.

c) The daily output of DNase I varies among individuals (in our work thus far about two to one) but is much more constant for a given individual. The hourly output is fairly constant in spite of very large differences in rate of urine production which are induced by forcing fluids or depriving subjects of fluids.

Significance to the program of the Institute: The work on normal human subjects is designed to serve as a baseline for studies on cancer patients.

Proposed course of project: Extension to cancer patients and to experimental animals. Further development of methods for accurately measuring the enzyme and inhibitor when both are present in the same system. Investigation of the source of urinary DNase.

Calendar Year 1956

Form No. CRP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-917
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,190	$4,440	$15,630	1/3	2/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	2/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI-917
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Shack, J.: Deoxyribonucleases of Mouse Tissues.
J. Biol. Chem. (in press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-918
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Research Development Projects in the Field of Electronics
PROJECT TITLE

7. Nathan Coffey
PRINCIPAL INVESTIGATOR

8. A. W. Pratt, J. C. Reid
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To design new electronic equipment as the needs of the Laboratory arise; to modify existing electronic equipment to meet the changes required in an experiment; and supervise the research development projects in the field of electronics.

Major Findings:

I. Modifications and Additions to Analog-to-Digital Converter and Printer--Dr. A. W. Pratt

A. An inherent fault in the commercial Analog-to-Digital Converter and Printer used to sense and record various conditions relating

(Use reverse and additional pages, if necessary)

to metabolism and Respiration Quotient of experimental animals was the frequent burn-out of certain solenoids which, while simple and cheap, resulted in the loss of important data while repairs were made. Soldered connections and the difficulty of mechanical alignment sometimes resulted in 30 minutes to one hour being required for the replacement.

Certain changes in the original circuit were made which reduced these burn-outs by more than 75%, and a mechanical improvement whereby automatic mechanical alignment and electrical plug-in were obtained was devised so that the now infrequent burned-out components could be replaced in a matter of minutes.

B. Another inherent fault of the equipment resulted in complete stoppage of data collection in the event of momentary or complete failure of certain sequential operations. An auxilliary sensing circuit, operating in time domain, was designed and added which permitted the instrument to skip over any inoperative component in the printer and to collect the balance of the data being fed to the machine, even though no operator was present.

C. A peculiarity of the Converter-Printer and sensing elements (transducers) made battery operation of the transducers mandatory when operating together. Characteristics of the transducers was such that even momentary removal of power to replace batteries resulted in loss of data collection for about thirty minutes. A switch-over panel was devised which permitted continuous operation with two sets of batteries, one being used while the other was charging, without even momentary loss of data collection.

II. Design and Development of Device to Permanently Record Curves of X-Ray Film Density Distribution at Pre-Set, Adjustable Levels--Mr. Robert Swain

A commercial densitometer had been incorporated into a device built by the Instrument Shop (Mr. Mencken) so that the densitometer reading head scanned the entire x-ray negative in a series of lines which progressed across the negative. As the density of the negative varied at the point being scanned, a panel meter gave continuous indication of the instantaneous density of the film. A device was requested which would mark a point on a sheet of graph paper corresponding to a pre-set density level each time this exact level was indicated by the panel meter, thus plotting the size and shape of the area receiving a given exposure. Such a device was developed and fitted in available space

on the composite instrument, and has been in use for several months, showing more than adequate sensitivity and accuracy, and has proved to be immune to extraneous noise signals. At the same time, the normal functions of the densitometer has not been impaired or affected.

III. Design and Construction of Bridge Circuit for Determining Small Changes of Temperature Affecting Environment of Experimental Animals-- Mr. W. C. White

In order to study small changes in temperature associated with energy expenditure in experimental rats, a bridge circuit utilizing thermistors as the sensing elements and constructed so that ambient temperature changes and/or air movement had negligible effect, was constructed. Both long- and short-term stability have been found to be excellent over the several months it has been in use. A sensitivity of 14 millivolts per degree Centigrade was developed.

IV. Re-Design and Construction of X-Ray Current Integrator (Long-Term)--Dr. C. R. Maxwell

During long x-ray exposures certain machines do not maintain constant current through the x-ray tubes. An auxilliary piece of equipment was requested which would present, in digital form, a number related to the time the machine was turned on and current which had flowed during that time, so that the exposure could be shortened or lengthened to compensate for any increase or decrease in desired current. Existing instruments for this purpose were examined and a new design developed which included "fail-safe" features, and which provided "On-Off" control of the x-ray machine, with choice of manual or automatic timer control.

V. Re-Design and Construction of Constant-Voltage Constant-Current Power Supply--Dr. J. C. Reid

For chromatography studies, investigator required house current (115 volts a.c.) to be converted to direct current at 0 to 1500 volts, stabilized at any selected intermediate value, or with the current stabilized at a selectable value. Existing circuitry was examined and a re-design made of the unit most nearly meeting requirements, so that all requirements of the investigator were met. This entirely new instrument has just been completed.

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-918
SERIAL NUMBER

VI. Partial Rebuilding of Mass Spectrometer--Mr. B. E. Burr

Failure of the manufacturer to incorporate a fuse in a minor circuit of the Mass Spectrometer resulted in a defective transformer being responsible for a near-fire and considerable damage to four units of the over-all instrument. Replacement of a number of components and almost complete rewiring of one of the units was necessary. In addition, the shut-down necessary while obtaining and installing new parts caused many weak points in remote sections of the instrument to break down. As a result, practically all of the control circuits had to be checked over and a number of weak components replaced. The instrument is now back in normal operation with "like-new" stability.

VII. Low Impedance Transistor Amplifier--Mr. Robert Swain

In proposed oxygen tension experiments, investigator advised that low-level, low-impedance direct-current amplifier would be required. Transistors seemed to be indicated, and since personal prior experience with them was limited, Bureau of Standards experts were consulted for data as to reliable manufacturers, newest circuit developments, etc.

Following this an amplifier was designed and constructed and placed in a test circuit. Operation was as predicted, but unfortunately Mr. Swain at this point had to discontinue the experiment due to lack of electrodes and the press of other duties.

VIII. Design and Construction of Calibrator to Permit Use of a Commercial Microammeter in Reading Current From an Ionization Chamber--Dr. M. Elkind

A specially designed ionization chamber for use in soft x-ray beam required provisions for reading ionization currents. A calibrator for use in conjunction with a commercial meter and the ionization chamber was built and used to accurately determine ionization currents.

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-918
SERIAL NUMBER

Significance to the program of the Institute: The ability to have these new types of electronic devices makes it possible to make metabolic analyses on normal and tumor-bearing animals which heretofore were made indirectly.

Proposed course of project: To develop new and finer adaptations for the measurement of metabolic changes; for example, development of automatic computation of the large volume of available data. This will require the construction of new gas-sensing elements having proper electrical characteristics compatible with the operating requirements of existing analog computers.

Calendar Year 1956

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-918
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,190	$4,440	$15,630	1	-	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	0	1	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-919
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Physiology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Development of Analytical Methods for Urinary Nucleotides and Congeners.
PROJECT TITLE

7. James C. Reid
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The purpose of this project is to determine whether or not there is a detectable urinary excretion of nucleotides or nucleosides, and if there is, to develop an analytical method for their identification and quantitative estimation. The method is also to be applicable to free pyrimidines, if any, and to free purines.

Methods Employed: Methods to be used are electrophoresis and chromatography.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd)

NCI-919
SERIAL NUMBER

Major Findings: A substantial activity connected with this project was a comprehensive review of the biochemical literature in search of clues to whether compounds of the type in question are in fact present in urine. A report embodying the results of the search and an estimate of the problem were prepared during the year. Among other things, it was indicated that an analytical method capable of a sensitivity of 1 - 0.1 parts per million will be required and this level has accordingly been established as a design requirement for the technique.

Experiments with a synthetic mixture of seventeen nucleosides, nucleotides, and free bases have shown that so far as solubility relationships are concerned these substances can be recovered from solution at the indicated dilution by mercury precipitation. Conditions have also been established which permit in principle their separation by double electrophoresis on paper.

Significance to the program of the Institute: This project is being pursued in collaboration with the Leukemia Service. Its ultimate aim is the accumulation of information concerning the mechanism of the therapeutic action of nucleotide analogues as well as the mechanism of their toxic side effects.

Proposed course of project: The fitting together of the two parts of the electrophoresis procedure presents some problems of technique which remain to be worked out. When this is accomplished there will remain the problem of dealing with the high concentrations of extraneous substances whose presence in urine complicates the preliminary isolation. This problem, which is the major one to be solved, will be approached by the use of stripping methods based on ion exchange resins.

Calendar Year 1956

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-919
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,460	$2,980	$10,440	1/3	1/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-920
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Cancer Physiology Section
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Identification of Compound "A-20"
PROJECT TITLE

7. James C. Reid
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: It is desired to identify what is believed to be a new amino acid found in rat liver.

Methods Employed: Chromatographic methods are used for isolation of the substance. The methods of physical and organic chemistry are used to accumulate information which will permit an identification to be made.

Major Findings: Information obtained so far appears to narrow the possibilities to one of two isomers. To decide between them will require the acquisition of larger amounts of material than have been available heretofore.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-920
SERIAL NUMBER

Significance to the program of the Institute: The substance being studied is presumably related to protein metabolism, which has basic importance in tumor growth.

Proposed course of project: An attempt will be made to devise a simplified isolation procedure and use it to acquire a reasonable supply of material. This will permit the application of chemical methods to effect a final determination of structure.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-920
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,460	$2,980	$10,440	1/3	1/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-921
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Energy Metabolism Section
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Increase in Induced Pulmonary Tumors in Mice Associated with Exposure to High Concentration of Oxygen
PROJECT TITLE

7. W. H. Heston and A. W. Pratt
PRINCIPAL INVESTIGATOR

8. ---
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

Project NCI-417, Laboratory of Biology.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

This project has been described by Dr. Heston (see Project NCI-417).

(Use reverse and additional pages, if necessary)

Calendar Year 1956

Form No. ORP-1
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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-921
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	0	0	0	-	-	-

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	0	0	0	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards and Publications

15. NCI-921
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Heston, W. E., and Pratt, A. W.: Increase in induced pulmonary tumors in mice associated with exposure to high concentration of oxygen. Proc. Soc. Exp. Biol. Med. 92: 451-454, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. ORP-1
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Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-922
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Physical Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Electron Microscopy of Tobacco Smoke.
PROJECT TITLE

7. H. Kahler
PRINCIPAL INVESTIGATOR

8. B. J. Lloyd, Jr.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None.

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: The object of this project was to make a survey of tobacco smoke by the technics of electron microscopy without any reference at the present time to carcinogenic activity.

Methods Employed: Cigars and cigarettes were smoked by a mechanical device which gives a standardized puff of known volume, duration and frequency. The smoke was then collected on microscope grids in the electrostatic precipitator, and examined in the electron microscope.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-922
SERIAL NUMBER

Major Findings: Nearly all the smoke is in the form of fluid droplets of great internal complexity in size from 1.5μ down to indefinitely small particles. It is estimated that the smoke from one cigarette coats about 0.01% of the alveolar surface with droplets. Certain differences were noted between cigar and cigarette smoke. The patterns formed from cigar smoke have a higher ratio of particulates to tarry material than cigarette smoke.

Significance to the program of the Institute: It seems probable that the physical properties of smoke will have a bearing on the lung tumor problem.

Proposed course of project: This research was intended to serve as an introduction to the subject and was of limited objectives. It may be best to leave further refinements in technic to such laboratories as are specializing in the tobacco problems.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-922
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,180	$4,440	$15,620	2/3	1/3	1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2/3	1/3	1	0

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NCI-922
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

H. Kahler and B. J. Lloyd, Jr.: The electron microscopy of tobacco smoke. J. Nat. Cancer Inst. In press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-923
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Physical Biology Section
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Electron Microscopy of Mouth Parts. Mosquitos Which Transmit Rabbit Fibroma Virus.
PROJECT TITLE

7. H. Kahler
PRINCIPAL INVESTIGATOR

8. B. J. Lloyd, Jr.
OTHER INVESTIGATORS

9. **IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).**

None.

10. **PROJECT DESCRIPTION (SEE INSTRUCTIONS):**

Objectives: The object of this work was to determine the mechanism of transmission of the rabbit fibroma virus by the mosquito. One theory is that the virus is simply absorbed on the mouth parts; a second alternative is that the virus is held internally and multiplies in the mosquito.

Methods Employed: Mosquitoes were fed on fibroma tumors on rabbits. The mouth parts were examined in the electron microscope after embedding and slicing.

Major Findings: So far no virus has been found on the mouth parts.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd): NCI-923
SERIAL NUMBER

Significance to the program of the Institute: It is of some importance to know whether the mosquito acts as a host in which the virus multiplies or simply acts as a mechanical transmitting agent.

Proposed course of project: Further examination of tissue sections from the mouth parts and other organs for possible sites of localization of the virus.

Calendar Year 1956

Form No. ORP-1
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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-923
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,450	$2,980	$10,430	2/3	-	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2/3	0	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NCI-924
SERIAL NUMBER

2. National Cancer Institute
INSTITUTE OR DIVISION

3. Laboratory of Physiology
LABORATORY, BRANCH, OR DEPARTMENT

4. Physical Biology Section
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Mechanism of Deoxyribonuclease Activation.
PROJECT TITLE

7. Joseph Shack
PRINCIPAL INVESTIGATOR

8. ----------
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

Objectives: To elucidate the chemistry of the activation of deoxyribonucleases by metallic cations.

Methods Employed: The action of deoxyribonucleases on deoxyribonucleic acid is determined as a function of pH, type and concentration of ions present, nucleic acid concentration, temperature, etc. by measurement of viscosity, pH, solubility and spectral absorption.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

10. PROJECT DESCRIPTION (Cont'd):

NCI-924
SERIAL NUMBER

Major Findings: The amount of Mg^{++} required to activate deoxyribonuclease I is constant above pH 6.5, increases between pH 6.5 and 5.5 and is again constant at a higher level below pH 5.5. The same result was obtained with the crystalline bovine pancreatic enzyme and with human urinary deoxyribonuclease I. Since nucleic acid does not exhibit titratable groups in the pH range where the above change occurs, it now appears likely that the enzyme as well as the substrate is directly concerned in the activation by Mg^{++}.

Significance to the program of the Institute: The deoxyribonucleases are valuable tools in study of nucleic acid structure, and perhaps have functions in the intracellular metabolism of these substances.

Proposed course of project: To continue with these studies of deoxyribonuclease I, and to extend the work to deoxyribonucease II, which is activated by both univalent and bivalent cations.

Form No. ORP-1
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(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NCI-924
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$7,460	$2,980	$10,440	1/3	1/3	2/3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1/3	1/3	2/3	0

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

NO ENTRIES FOR 15, 16 AND 17.

LABORATORY OF PHYSIOLOGY

National Cancer Institute

Calendar Year 1956

Papers published since the last annual report from projects which have been completed and are not reported this year:

(1) Loomis, L. N., and Pratt, A. W.: The histogenesis of Rous Sarcoma I induced by partially purified virus. J. Nat. Cancer Inst. 17: 101-121, 1956. (Project Report of Calendar Year 1952 entitled "Observations on the pathogenesis of Rous sarcoma I").

(2) Putney, F. K., and Pratt, A. W.: X-irradiation of L-malic acid in aqueous solutions. Radiation Res. 5: 134-145, 1956. (Project Report Serial No. 913-Calendar Year 1955).

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. 1200
SERIAL NUMBER

2. NCI
INSTITUTE OR DIVISION

3. CCNSC
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. CANCER CHEMOTHERAPY
PROJECT TITLE

7. K. M. ENDICOTT
PRINCIPAL INVESTIGATOR

8. J. LEITER, C. HUTTRER, M. SHIMKIN
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

The search for a successful chemical treatment of cancer has received much attention by Congress in the past several years. Sparked by the interest and support of Congress, the National Cancer Institute has developed a coordinated national program of research jointly sponsored by four Federal and two

private agencies. An inter-agency staff organization - the Cancer Chemotherapy National Service Center - is now in its second year of operation as the clearing house, expediter and coordinator of hundreds of interrelated research projects being carried on throughout the Nation in universities, hospitals, government laboratories and private industry.

These projects in one way or another all aim at the discovery of a substance which, like penicillin in bacterial disease, will have the property of attacking cancer cells without seriously harming the normal cells of the body. There is already abundant evidence that several different types of chemical agents, when given by mouth or by injection to cancer patients, are capable of causing spectacular, though temporary, regression or disappearance of widespread cancer lesions. Unfortunately, all of these chemicals have undesirable side-effects on normal tissues and most of them are highly toxic to one or more types of essential normal cells such as the blood-forming cells or the cells which line the intestinal tract.

Thus far the "margin of safety" has been so small that it is not possible to destroy all of the cancer cells. The surviving cancer cells multiply and eventually kill the patient. It is our hope that through intensive efforts we may find chemicals which are much more toxic to the cancer cell and much less toxic to normal cells so that permanent cures may result.

The search for better chemicals is largely an empirical program of successive steps of "trial and error" screening. For obvious reasons, the number of chemicals which can be tested against human cancers is quite small. Yet there are literally millions of synthetic and natural chemicals to choose from. The chemotherapist must therefore devise a screening system for selecting those few chemicals which he may try in man. His constant dilemma is that until he discovers a cure he has no assurance that the screening method is the proper one.

During the past year the cancer chemotherapy program has been moving forward rapidly.

The increase in appropriations for Fiscal Year 1957 has permitted the CCNSC to initiate additional activities. Training grants have been made to a number of institutions for the training of personnel in clinical investigation, pharmacology, steroid biology and biochemistry, etc. Contracts have been let for hormone assays, the level of activity in the screening program has been increased sharply, and a major program in the antibiotic field is in the making. This major expansion in program activities has not been without its problems, both administrative and technical. We have had to reorganize and expand the

staff of the Service Center to a level of approximately 60 persons. The Center is now divided into three program areas with an Assistant Chief for each area. Dr. Leiter is Assistant Chief for Laboratory Operations, Dr. Shimkin for Clinical Operations, and Dr. Huttrer for Program Analysis, Documentation, and Informational Activities. Much attention has been given to the problems of administration of the contract program, and questions surrounding the knotty problem of contracts vs. grants seem to have been reasonably resolved, at least for the time being.

The major elements of the recommendations of the National Committee to the National Advisory Cancer Council regarding its policy on evaluation of cancer cures have been recommended by the Council and adopted by the Surgeon General.

Perhaps the most significant development in the past six months has been the response of the pharmaceutical industry to the cancer chemotherapy program. From our discussions with individual companies, with the Industry Subcommittee, and with several committees of the American Drug Manufacturers Association, it is clear that we can count on a much greater program in industry than had previously been expected. Materials are now flowing from industry to the Center for screening at a rate of approximately 25,000 per year and the demand has not yet been met even so. In addition, a number of companies have been discussing with the Service Center the possibility of entering into contracts with the Center for a wide variety of activities to be carried out in company facilities. A number of the problems relating to such programs are touched upon below in the report of the activities of the Industry Subcommittee. For full implementation of such a program it may be necessary for the Department of Health, Education, and Welfare to seek some form of authority for entering into multiple year contracts and for establishing patent policy guidelines to meet the special problems in this area. The remainder of this report will consist of summaries of the activities of the Industry Subcommittee, activities of the several Panels, and documentation activities at the Service Center.

INDUSTRY SUBCOMMITTEE ACTIVITIES

During the past year the Industry Subcommittee has held three meetings and its special Atlantic City meeting for executives of the chemical and pharmaceutical industry. The Committee meetings on June 20 and in the afternoon of September 17th were primarily concerned with the plans and final details for the session held at Atlantic City, concurrently with the Fall meeting of the American Chemical Society, when nearly one hundred research executives on invitation of the Industry Subcommittee listened to a program consisting of the following:

I. Introductory Remarks, Dr. M. L. Moore

II. Purposes and Policies of the National Cancer Chemotherapy Program, Dr. Sidney Farber.

III. Procedures and Operation of the Program as Implemented by the Cancer Chemotherapy National Service Center, Dr. K. M. Endicott.

At the end of these presentations there was an informal discussion period. Members of the Committee assisted the chairman in answering questions. Mr. Fowler, counsel for the Committee, clarified many points involving the patent problems involved.

A major objective of the Cancer Chemotherapy National Program is the obtaining of chemicals from industry and the testing of these for antitumor activity. The Industry Subcommittee has been a major factor in the evolution of the program, culminating in the present state of cooperative effort whereby industry is currently submitting for antitumor screening more than 8,000 synthetic compounds annually and, in addition, is supplying for test 17,000 culture filtrates annually. Even a higher rate of submission can be anticipated in another year.

Since the beginning, the Industry Subcommittee has recognized the importance of close cooperation with such organizations as the American Drug Manufacturers Association, the American Pharmaceutical Manufacturers Association, and the Manufacturing Chemists Association. In the preparation of the list of invitation to the September meeting, the "official correspondents" and membership lists of these three organizations were employed. The wisdom of these actions may be seen already. The committee of the A.D.M.A. on National Research Council and National Institutes of Health Research now has appointed an advisory group to meet with them. On this new group are the chairman of the Industry Subcommittee and the Industry Representative of this National Committee. We now anticipate that Drs. Moore and Sweet can be extremely helpful to the A.D.M.A. Committee and that the experience gained from the Industry Subcommittee will be useful in consideration of the cancer program.

The present program will, of course, bring about a general increase in clinical research personnel and expanded facilities for clinical cancer chemotherapy. It is anticipated that industrial cooperation will result in more compounds for clinical evaluation. In the future, with increased activity in the general field of clinical cancer chemotherapy, it should be increasingly easy for both industry and the Center to have their compounds carefully evaluated in man. In order to head off any concern about a bottleneck in clinical investigation, the National Committee may wish to make a statement to the effect that it is concerned with seeing that prompt clinical testing is available for all compounds, whether of industrial or academic origin.

The Industry Subcommittee has been working on the problem of patents. In particular, it has considered the special situation

arising in the hormone and steroid field. It is also currently concerned with advising the Center how best to establish a program under its contract authority which will enlist the maximum participation from industry. The Committee has emphasized that in making its contracts with industry, the Center must at all times consider all contracts with industry as an interrelated problem so that one contract is not more favorable to the recipient industrial organization than other contracts previously or subsequently awarded. Also, the opportunity for holding contracts must be extended equally to all interested industrial laboratories.

The Committee has also, after deliberations on the subject, expressed the belief that industry might utilize government financial support for biological evaluation of chemical compounds. Since no great patent problem is involved, industry could carry out with government support such operations as screening, toxicological studies and pharmacology. However, it has advised that drug development programs would be far more complicated. With respect to these, it noted that through government supervision of such efforts a directed program might evolve and that such should be carefully avoided. At its recent meeting the Committee also advised that there should be some hesitancy for industry going into a research program of chemical synthesis under government support, where such involved the originating of new compounds as distinguished from the production of batches of chemicals for experimental or clinical testing.

The Committee has continually urged the Center to establish its program in such a way that the various component members of the chemical and pharmaceutical industry would become more interested in the cancer problem through contact with the screening operation and then, as the chemists and biologists became better acquainted, a developmental program involving synthesis might evolve.

The role of the Industry Subcommittee is possibly well demonstrated by the discussions that have taken place at its meetings on the subject of the publication of what has been called "Negative Data" in antitumor screening. Several years ago, in order to eliminate possible duplication of laboratory effort under grant-in-aid support, a group of chemotherapists were asked to collate and publish information on work which, because of the lack of a positive and useful result, would not otherwise find its way into the cancer literature. Early in 1955 at a meeting of the Industry Subcommittee it was pointed out that such a mention of a chemical compound in print constituted an official disclosure which, after the lapse of one year, eliminated the possibility of obtaining patent protection on the compound as a new product. After recurrent discussion it was finally agreed at the past December meeting that the Center should be informed that it was the opinion of the Committee that the use of public funds to defray the cost of publication of future collections of such "Negative Data" was

not only a waste of public funds but might even be detrimental to the cancer program. Thus, they have recommended the resolution of a problem that has long been with us. Similarly, on other matters the Center seeks and gets advice through the discussion of topics which it brings before the Industry Subcommittee. Frequently this is in the form of a consensus of opinion rather than a complete resolution.

The Industry Subcommittee is a dynamic group of public-spirited citizens who are most able in their professional fields. It is anticipated that they will continue to serve the program well. Toward that end, the Committee has been set up on the basis of four-year term appointments which are rotated to provide the annual inclusion of two new members to the roster of eight.

The Committee has frequently discussed the enlargement of its size so as to include more representation for all members of industry. However, in order to maintain it as a dynamic discussion group, it is felt such an increase in size is not desirable. There is now underway some plan for organizational liaison which should effect a wider industry participation in the Cancer Chemotherapy National Program.

CHEMISTRY ACTIVITIES

Procurement of Chemicals for Screening

Approximately 7,000 chemical compounds have been procured for the screening program. This brings the total for the first year of operation to 12,000. More and more arrangements are being made with pharmaceutical companies for the screening of their compounds on a confidential basis. These now constitute over half of the total being received. Visits by the staff have been made to 16 universities, 4 non-profit institutions, and to 6 industrial installations to discuss cancer chemotherapy. Trips are scheduled for the next few months to about 10 additional institutions.

In addition to procurement of chemicals for screening, the Center has purchased chemicals of particular interest to investigators in cancer chemotherapy. These include chemicals used as intermediates for synthesis, compounds undergoing clinical trial, etc.

Chemical Synthesis Contracts

The Panel recommended approval of contracts for the synthesis of compounds of interest to cancer chemotherapy with the Stanford Research Institute, Southern Research Institute, Monadnock Research Institute, Radiological Research Institute, New Mexico Highlands University, Pennsylvania State University, and California Institute of Technology.

Drugs Standards Subcommittee

This subcommittee draws up specifications for chemicals being procured for human evaluation studies. Since June, specifications have been formulated for chloroquine nitrogen mustard dihydrochloride and pamoate, 6-chloropurine, and DON.

Analytical Subcommittee

No meetings of the subcommittee were held. Analytical service laboratories are now in operation at George Washington University (H. George Mandel), the University of Michigan (Lauren A. Woods), and the University of Wisconsin (Gilbert J. Mannering and F. E. Shideman).

SCREENING ACTIVITIES

The first half of 1956 represented a "tooling up" period as far as screening activities of the Service Center were concerned. Several laboratories under contract with the Center developed facilities for large-scale evaluation of agents against a spectrum of three mouse tumors.

The latter half of the year has been devoted primarily to the problem of putting these facilities to work in extending the base of screening to a level of over 50,000 tests a year. Following the establishment of a suitable policy for handling drugs from industry, active negotiations have resulted in a flow of 6,000-8,000 synthetics per year and approximately 17,000 antibiotic culture filtrates a year from these sources.

Because of the shortage of inbred mice, testing of antibiotic filtrates has been restricted to one tumor, Sarcoma 180. However, the high proportion of active cultures in this short period of six months has made it desirable to extend the evaluation of filtrates to the other tumor systems. Accordingly, the Center has arranged for the production of about 35,000 breeder-mice in the next few months. This will make possible the increased production of inbred hybrids from 250,000 per year to almost 1,000,000 per year by the Fall of 1957 and will enable the Center to test filtrates against all three tumors in its screen. This extension will require a doubling of screening capacity to a level of at least 100,000 tests a year even if no additional arrangements for materials are made.

At least a dozen materials, principally antibiotic filtrates, appear to show sufficient antitumor activity so that the pharmaceutical companies furnishing them have begun some fractionation. One compound has been scheduled for preclinical pharmacology.

In other areas, pilot studies of microbiological systems including tissue culture, are virtually completed. Reports of these studies are being analyzed. The Screening Panel has recommended the establishment of a tissue culture screen at a level of about 5,000 materials to be run in parallel with the mouse screening.

The Screening Panel has continued to give active consideration toward the developments of other screening methods, in vivo as well as in vitro. A study of ascites tumors is about to be initiated under contract at the University of California. The Panel is considering proposals for specialized screening programs such as lymphomas, drug-resistant tumors, combination therapy, etc.

The Screening Panel has also given serious thought to problems engendered by the tremendous increase in laboratory animal production. It has sponsored a study by the Institute of Laboratory Animal Resources of the National Research Council to develop criteria for the certification and accreditation of animal breeders and for the development of adequate genetic control over the commercial production of inbred animals. The Panel has also been concerned about the dangers of the spread of disease resulting from the great increases in mouse production and is establishing pilot diagnostic laboratories for detecting ectromelia and salmonella as well as other laboratory animal diseases.

ENDOCRINOLOGY ACTIVITIES

Activities in the area of endocrinology have been initiated during this past year. Under the guidance of the Endocrinology Panel, the basic organization of three areas of activity have been developed. These are:

Hormone Assay Activities

Preliminary procedures for 14 basic assays have been developed by an Assay Subcommittee. These are designed to furnish a profile of androgenic, estrogenic, progestational, and corticoid activities of steroids. These profiles are to be correlated with the therapeutic activity of the hormones against different types of human and animal tumors.

Contracts with two laboratories have been in effect since November 1. These laboratories are in the process of evaluating the actual assay procedures for a group of 37 representative steroids and for about two dozen similar steroids recommended for clinical trial by the Endocrinology Panel.

The Service Center and the Endocrinology Panel are developing arrangements for making the assay facilities available on a broad scale to suppliers of steroids. As the demand for these facilities develops, expansion of the Service Center's activities will be made.

Clinical Activities

Through the activities of a Clinical Subcommittee of the Endocrinology Panel, broad study of the effects of about 24 androgens is about to get underway. A suitable protocol has been developed for the evaluation of these steroids in comparison with testosterone propionate. Procurement of large quantities of these steroids is virtually completed. In several instances toxicological and pharmacological data are necessary before the steroids may be safely administered. Contracts for such studies are in the process of being set up and will be undertaken whenever the compounds become available.

Experimental Tumor Studies

Representative steroids are being made available to Dr. Stock of the Sloan-Kettering Institute for evaluation against a spectrum of animal tumors. Similarly, steroids submitted for assay and for clinical studies are being screened in the Service Center's screening program whenever sufficient material is available. Additional studies in other types of experimental human systems are underway. The Panel and staff have been actively surveying the possibilities for developing experimental tumors sensitive to steroids. Such developmental programs are under consideration by the Endocrinology Panel. At its November meeting, the Panel sponsored a small symposium on endocrine-responsive tumors in experimental animals.

PHARMACOLOGY-BIOCHEMISTRY ACTIVITIES

Preclinical Pharmacology Studies

A pharmacological and toxicological report on one compound (CB-1348) was received from the Food and Drug Administration. When a suitable form for reporting such data is developed, this report as well as subsequent ones will be circulated and published in "Current Research" or in an appropriate scientific journal.

Four additional compounds, including one developed in the screening program, have been submitted for preclinical pharmacology. Pharmacological data available for three other compounds were considered adequate. The Pharmacology-Biochemistry Panel also recommended that the Service Center set up suitable pharmacologic studies of combinations of drugs used clinically. It also recommended that suitable carcinogenic studies be made of all agents being used clinically.

Physiological Disposition of Drug Studies

As a result of stimulation by the Pharmacology-Biochemistry Panel and of the Chemistry Panel, three laboratories, suggested by

research grants, have been established to study the physiological disposition of compounds recommended for preclinical pharmacological studies. These laboratories are located at the University of Michigan, George Washington University, and at the University of Wisconsin. These laboratories have agreed to have their work coordinated by the Cancer Chemotherapy National Service Center. Compounds specifically recommended for these studies were 4-(p-dimethylaminostyryl) quinoline and diethyldioxystilbene diphosphate. Studies in all three laboratories are expected to get underway in the Spring of 1957.

Other Activities

A proposal for the large-scale preparation and subsequent screening of enzymes was considered by the Panel. This was presented to the National Committee by Dr. Haddow at the June meeting. The Panel recommended that development of this area of research by the Center be deferred until more definitive data became available.

The Panel recommended scientific approval of a contract proposal from Southern Research Institute for biochemical studies on the mechanism of action of anticancer agents. Clarification of the relationship between research grants and research contracts was requested by the Panel.

In order to develop new ideas and approaches in the area of cancer chemotherapy, the Pharmacology-Biochemistry Panel devoted the major part of two of the three meetings held since July to an informal scientific discussion by Panel members and invited participants. A digest and analysis of this discussion has been prepared. The implementation of novel ideas proposed at these sessions is being considered by the Panel.

CLINICAL STUDIES ACTIVITIES

The Clinical program has rapidly expanded to the point where there are now more than 75 institutions participating in cooperative study groups. A clinical study has been organized in cooperation with the Endocrinology Panel. One cooperative group is already functioning and will evaluate experimental androgens and progestational substances in the therapy of far advanced breast cancer. Another group is currently being formed to evaluate estrogens in the therapy of breast cancer. An initial meeting has been held to organize a group to study the effects of steroids on prostatic carcinoma. Additional groups are contemplated for the purpose of testing certain steroids in the treatment of carcinoma of the cervix and lung.

A group of surgeons and radiologists met to discuss the use of isotopes and chemotherapeutic agents as adjuncts in the definitive surgical and radiological treatment of cancer. A cooperative study group has been formed and protocols are being prepared. These

investigators initially plan to study gastrointestinal malignancies and expect later on to include breast, lung, and ovarian tumors.

A Cancer End Results Committee has been organized with Clinical Studies Panel sponsorship and is composed chiefly of tumor clinic registrars from state, municipal, and institutional registries. It is clear to most people that the evaluation of chemotherapy has to be related to the end results achieved by other means of treatment, and therefore there is need for a research program based on long-term follow-up of cancer patients. This committee has held two meetings, and it is hoped that the participating members will soon be ready to begin operations.

Close liaison with the Veterans Administration has been maintained, and a parallel program has been developed in Veterans Administration Hospitals. The V. A. participants have been enthusiastic in their willingness to cooperate, and with the vast facilities of the V. A. system available, the magnitude of the overall program will be considerably increased.

DOCUMENTATION ACTIVITIES

The Cancer Chemotherapy Bibliography appeared about December 1, as Part II of the November issue of Cancer Research. Forty-five hundred copies were printed and distributed as follows: Cancer Research subscribers, 2700; National Library of Medicine, 500; Cancer Chemotherapy National Service Center, 1300. About 400 of the CCNSC copies have been distributed to interested libraries and individuals. The bibliography was compiled for the Service Center by the National Library of Medicine. The Center is continuing to keep the bibliographic data up to date. It is also processing and filing the photostats of the complete articles noted in the bibliography. In the near future these will be available for use in answering inquiries.

A peek-a-boo index of the chemicals is being considered. The punch necessary for this system has been built by the National Bureau of Standards and the index will be started as soon as special plastic cards are delivered. This will make possible searches for compounds having one or several desired structural characteristics.

A literature search was made for information on the anticancer tests and mammalian toxicity of about 200 compounds being used in the evaluation of tissue culture and microbiological systems as screening methods. The pertinent tests of these compounds were tabulated and distributed to those cooperating in this evaluation study.

INFORMATION ACTIVITIES

In the information area, during the past year a lantern slide file of approximately 70 slides has been set up for use as illustrative material for lecture purposes. The file includes organization charts of the chemotherapy program, the screening operation, and chemical formulae. Individual sets of selected slides have been assembled for the personal use of certain investigators who have indicated a constant need for such slides.

A number of newspapers and publications have shown interest in the program and have been given information on program developments. Other information outlets have been contacted for the publication of news items. Articles have appeared in the Saturday Evening Post and Colliers, and should appear in two other national magazines.

A speech outlining the organization of the national program was delivered by Dr. K. M. Endicott at an international symposium on chemotherapy of cancer held in Oslo, Norway, and will be printed in Acta Unio Internationalis Contra Cancrum. In addition, Dr. J. R. Heller addressed the Faculty of Medicine at the University of Buenos Aires explaining the national program and citing some of the problems facing investigators. This speech will also be published.

A brochure summarizing the national program and suggesting ways in which industry can assist in the national program was written for and distributed to representatives of the chemical and pharmaceutical industry.

Efforts have been made to develop Current Research in Cancer Chemotherapy into an effective means of exchanging and disseminating information on cancer chemotherapy and on the national program. An editorial policy has been developed and an editorial board appointed. Material included will cover all phases of cancer chemotherapy, and will be in the form of progress reports of current research, communications, screening data, announcements, abstracts, and reports from interested investigators, the Cancer Chemotherapy National Service Center staff, and its technical panels. The mailing list is now 1,500 and six issues have been printed to date.

An exhibit panel on the national program is being developed for use at scientific meetings.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. 1200
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$3,943,300	$75,320	$4,018,620	22	36	58

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	24	40	36	0

13. BUDGET ACTIVITY:

RESEARCH	☐	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S):

(No entries for 15, 16 and 17)

(Use reverse and additional pages, if necessary)

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